Remarks

Claims 1-58 are pending in the application. Claims 13-58 are withdrawn from consideration pursuant to a restriction requirement. Claims 1-12 have been rejected.

Response to the Objection to the Specification for Alleged New Matter

The examiner contends that the amendment to the specification entered the typographical error as "mg/kg/day" to "mg/day" impermissibly added new matter. The examiner contends that the specification does not support this change because the examples provided by the applicants in the specification (p. 47-49) gave does in mg/kg and that the doses were given in one dose, which the examiner equates with "per day".

An amendment to correct an obvious error does not constitute new matter where one skilled in the art would recognize the existence of error in the specification and the appropriate correction. MPEP 2163.07 (citing *In re Odd*, 443 F.2d 1200 (CCPA 1971)).

Here the person skilled in the art would easily recognize the existence of the error because the skilled person would appreciate that the dose ranges as stated (in "mg/kg/day") would result in grossly excessive doses. For example, the recited range of 100 to 1500 "mg/kg/day" would require a dose in the range from about 7g to 105g for an average (70kg) human being. A standard prescription for a one month supply at 1500 mg/kg/day for an average person would weigh 7 pounds. It is respectfully submitted that such doses would be recognized as extremely high and clearly erroneous by the person skilled in the art. The person skilled in the art would also immediately recognize a reasonable dose range for human would be obtained that if the "per kg" notation were recognized as an error, and thus immediately recognize this as the appropriate correction.

It is clear that the description on page 36 is clearly intended to describe doses suitable for human administration. It is well known in the art that suitable doses for a given compound vary across species, and that the suitable dose does not vary linearly according to body weight. Thus, there is no mg or mg/kg dose range that can be generally stated to be suitable across different species. The fact that p. 36 refers to suitable doses for treatment of a "patient", states that the dose may vary with size, weight, age and sex, but does not

mention the possibility of variation with species would clearly indicate to the person skilled in the art that dose ranges suitable for treatment of a particular species, i.e. human, were being described.

Example 6 on pages 47-49, cited by the examiner to show that the correction is not supported by the specification, in fact clearly indicates that the reference to 100 to 1500 mg/kg/day doses on page 36 is an error. The range of doses given to mice in Example 6 (15-60 mg/kg with doses in the range 30-60 mg/kg giving an effect) is completely different from the ranges which are indicated on page 36 as being desirable and preferred to use (if the doses are understood as being in "mg/kg/day"). This, coupled with the fact that the doses indicated on page 36 (read as "mg/kg/day") are excessively high, further serves to make the error in the dose units provided on page 36 clearly apparent. The error is even clearer when it is considered, as is well known in the art, that a human equivalent mg/kg dose is much lower than a corresponding mouse mg/kg dose.

Taking into account the well known fact that a human equivalent mg/kg dose is much lower than a corresponding mouse mg/kg dose, it may be seen that Example 6 is in fact fully consistent with the requested correction of mg/kg/day to mg/day as the unit for the doses on page 36. It is recognized in the art that the relationship between effective doses for drugs across species is complex and not linear according to body weight. However, an estimate of a human equivalent dose can be derived for conversion of animal by application of a interspecies scaling factor. Although there is no universally agreed scaling factor, scaling factors based on body surface area or $(\text{body mass})^{3/4}$ are recognized as being useful "rule of thumb" measures. These lead to a human equivalent mg/kg dose about 12-fold (for body surface area scaling) or about 7-fold (for ¾–power scaling) less than a corresponding mouse dose.[1] Applying these scaling factors to the 15-60 mg/kg range used for the mouse studies in Example 6 give human dose ranges of about 1.25-5mg/kg or about 2.1-8.5mg/kg using body surface area scaling and ¾-power scaling respectively, which translate,

[1] DeVita, Cancer: Principles and Practice of Oncology, 3rd Ed. (1989) (p. 293, Table 16-7); Lorenz R. Rhomberg, *et al.*, "Method for Identifying A Default Cross-Species Scaling Factor", Report prepared for the EPA, Available online at oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=455644 (April 2, 2004) (p. 2 and Appendix). While the latter from after the priority date of the present application, the articles are not original research references and are believed largely to reflect information known at the time of filing the application.

respectively, to doses of 87.5- 350mg and 150-600mg for an average 70kg human being, fully consistent with the correction made on page 36 where the preferred dose as corrected is 100mg to 500mg per day.

Since both the nature of the error and the appropriate correction would be apparent to the person skilled in the art, the amendment made to the specification does not constitute new matter and therefore cancellation of the correction is not required. The examiner is respectfully requested to reconsider the objection to the specification in view of applicants comments set forth above.

Response to the Provisional Rejection of Claims 1-12 For Obviousness-Type Double Patenting

The examiner has provisionally rejected claims 1-12 on the ground of non-statutory obviousness-type double patenting over claims 1, 10-14 and 16-20 of co-pending Application No. 10/578,522.

Since the rejection is provisional, the applicants respectfully request that the rejection be held in abeyance until agreement as to the patentability of the claims is reached.

Response to the Rejection of Claims 1-12 Under 35 U.S.C. § 103(a) Over Kórósi in view of Ito

The examiner has rejected claims 1-12 under 35 U.S.C. § 103(a) as allegedly unpatentable for obviousness over Kórósi, *et al.*, U.S. Patent No. 4,322,346 ("Kórósi") in view of Ito, *Tokyo Ika Daigaku Zasshi*, **1981**, *39*, 269-384 ("Ito"). The applicants respectfully traverse.

The examiner states that applicants' specific compound can be derived from Kórósi by appropriate selection of options from the numerous options presented in Kórósi. The examiner states that both Kórósi and Ito teach a regioisomer of the applicants' compound. The examiner claims that substituting the methoxy groups in the 7 and/or 8 positions of the 2,3-benzodiazepine ring with a hydroxyl group brought about a decrease in the acute toxicity and had similar effects to tofisopam, and that the methoxy group at the 7 position of

the 2,3-benzodiazipine ring plays the most important role in the activity of tofisopam. The examiner states "a novel useful compound that is isomeric with the prior art compound is unpatentable unless it possesses some unobvious or unexpected beneficial property not possessed by the prior art compound" (citing *In re Norris*, 179 F.2d 970, 84 (C.C.P.A. 1950)).[2] The examiner further states that it would have been obvious for the person skilled in the art to formulate an (R) enantiomer or (S) enantiomer because it is known that it is possible to separate enantiomers, and that the person skilled in the art would expect the enantiomers to have substantially different pharmacological activity. The examiner also states that it would be obvious to formulate the optical isomers with specific levels of optical activity because optimizing a result-effective variable is within the skill of the art.

In order to establish *prima facie* case of obviousness, three basic criteria must be met:

(1) A suggestion or motivation to modify the reference or to combine reference teachings.

(2) A reasonable expectation of success.

(3) The reference (or references when combined) must teach or suggest all the claim limitations.

MPEP 2142. As the MPEP further notes, "[t]he teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *Id.* (citing *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991)).

The examiner appears in the present rejection to rely principally on a *per se* rule that purportedly states that "a novel useful compound that is isomeric with the prior art compound is unpatentable unless it possesses some unobvious or unexpected beneficial property not possessed by the prior art compound." The examiner cites *In re Norris*, 179 F.2d 970, 84 (C.C.P.A. 1950), to support this proposition. However, the Federal Circuit has expressly held that there is no such thing as the *per se* obviousness suggested by the examiner:

[2] The examiner incorrectly cites this case as dating from 1970.

> The use of per se rules...flouts section 103 and the fundamental case law applying it. [R]eliance on per se rules of obviousness is legally incorrect and must cease. [O]ur precedents do not establish any per se rules of obviousness.... Any conflicts as may be perceived to exist derive from an impermissible effort to extract per se rules from decisions that disavow precisely such extraction.

In re Ochiai, 71 F.3d. 1565, 1572 (Fed. Cir. 1995).

The *Norris* case cited by the examiner dates from 1950 (not 1970 as stated by the examiner). The examiner will find that the case is not cited in the MPEP. The case dates from prior to the enactment of the present Patent Act which introduced the statutory definition of obviousness in § 103 and the *Graham* decision setting forth a standard for evaluating obviousness. According to applicants' research, the *Norris* case cited by the examiner has not been cited in any judicial or Board of Patent Appeals and Interferences opinion for over 40 years.[3] The analysis of *Norris* has clearly been displaced by the analysis of obviousness under *Graham*, including the concept of *prima facie* obviousness. The Federal Circuit has mandated that *Graham* findings must be made "in *every* case to establish a prima facie case of obviousness." *Ortho McNeil Pharm., Inc. v. Mylan Labs., Inc.*, 348 F. Supp. 2d 713, 749 n.19 (citing *In re Mayne*, 104 F.3d 1339, 1341 (Fed. Cir.1997) (emphasis in original). In addition, the courts have noted that "generalization is to be avoided insofar as specific structures are alleged to be prima facie obvious one from the other." *In re Jones*, 958 F.2d 347, 350 (Fed. Cir. 1992) (citing *In re Grabiak*, 769 F.2d 729, 731 (Fed. Cir. 1985)).

It is acknowledged that the examiner appears to have attempted to set forth a rationale for a suggestion or motivation to combine or modify the reference teachings. However, it is respectfully submitted that a fair reading of both the references and the examiner's grounds of rejection show that the rejection is based on a selective reading of the references guided by applicants' claims rather than by any teaching of the references. If so, this would not be proper: in making an obviousness determination, the MPEP requires that the references "must be considered as a whole and must suggest the desirability and thus the obviousness of making the combination" and "must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention." MPEP § 2141 (II).

Viewed as a whole, the Ito reference clearly would not have provided the motivation suggested by the examiner to make the 8-hydroxy compound of which pharmaceutical compositions are presently claimed. As the examiner characterizes Ito, the substitution of hydroxyl for the methoxy groups of tofisopam is represented as being desirable. The examiner states that substituting the "methoxy groups in the 7 and/or 8 positions of the 2,3-benzodiazepine ring with a hydroxyl group ... brought about a decrease in its acute toxicity and had similar effects". The examiner therefore implies that Ito teaches replacing methoxy in tofisopam as a desirable modification because toxicity would be lowered and the desirable pharmacological activity of tofisopam retained. The examiner therefore contends that making the 8-hydroxy derivative specifically would have been desirable due to the existence of the regioisomer.

It is respectfully submitted that the examiner mischaracterizes the teachings of Ito. Contrary to what is implied by the examiner, substitution of methoxy for hydroxy did not improve the properties of tofisopam. While acute toxicity was reduced, so also were the desirable pharmacological properties. This led Ito to conclude that the methoxy groups were important to the pharmacological properties of tofisopam. This is supported, for example, by the abstract of Ito, which states:

> "[T]he relationship between the chemical structure and pharmacological activity of [tofisopam] was studied. Substituting the hydroxyl group for the methoxy group in the 7 and 8-positions of the benzodiazepine ring and 3 and 4-position of benzene ring of [tofisopam] brought about a decrease in its acute toxicity *and all psychotropic activities*. Anti-noradrenaline activities of these related compounds were equivalent or *inferior to [tofisopam]*. These results suggest that *the methoxy group [sic] in the chemical structure of [tofisopam] are intrinsically related to its pharmacological activities*.

(emphasis added). Ito therefore did not suggest that substitution of hydroxy would result in improved compounds as suggested by the examiner. Rather, Ito teaches the *importance* of the methoxy groups to the pharmacological properties of tofisopam, and therefore teaches away from replacing methoxy groups with hydroxyl. Ito therefore teaches away from making the pharmaceutical compositions of the 8-hydroxy compound as presently claimed.

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[3] Applicants performed a search of the case using Shepards on the Lexis database.

The examiner's rationale as to why it would have been obvious for the person skilled in the art to make specifically the 8-hydroxy compound is also unclear, and appears to rest solely upon the alleged similarity to the regioisomeric 7-hydroxy compound. Apart from the incantation of the non-existent *per se* rule, the examiner provides no reasons why the person skilled in the art would have been motivated to modify the reference to make specifically the 8-hydroxy compound as claimed in the compositions of the present invention.

The data in Table 10 of Ito refute the examiner's suggestion that the 7-hydroxy and 8-hydroxy compounds should have been expected to have similar pharmacological properties because they are regioisomers. Compounds TF1 and TF2 in Ito are regioisomers of each other, yet they appear from Table 10 of Ito to have pharmacological properties that are completely different from each other. Compound TF1 completely lacked activity in inhibiting aggression and inhibiting muricide, but retained anti-noradrenergic properties comparable to tofisopam. On the other hand, in the isomeric compound, TF2, the 7-hydroxy compound, anti-noradrenergic properties were completely abolished, while modest activity in inihibiting muricide was retained. Thus, the pharmacological properties of hydroxy analogues of tofisopam appear from Table 10 to be exquisitely dependent on the position of the hydroxyl group. Accordingly, nothing about the pharmacological activity of the claimed compositions comprising the 8-hydroxy compound could have been predicted merely from knowledge of the properties of the 7-hydroxy compound disclosed in Ito.

Further, even if it were assumed, arguendo, that the 8-hydroxy compound would have been expected to show similar properties to the 7-hydroxy compound, based upon the properties of the 7-hydroxy compound as reported in Ito, there would have been no motivation for the person skilled in the art to modify Ito to make the 8-hydroxy compound. As Table 10 of Ito .shows, the properties of the 7-hydroxy compound are markedly inferior to tofisopam, and are also inferior to several of the other hydroxyl compounds tested. It was for this reason that Ito concluded that the 7-methoxy group was especially important to the pharmacological properties of tofisopam (a conclusion pointed out specifically by the examiner). The 7-hydroxy compound ("TF2") was completely inactive in inhibiting aggression, was only partially effective – less effective than tofisopam – in inhibiting muricide, and completely lacked anti-noradrenergic activity. The weak antimuricidal

activity is, in fact, the only pharmacologic activity shown for the 7-hydroxy compound. It is respectfully submitted that the lack of activity observed for the 7-hydroxy compound as reported Ito would teach the person skilled from making further compounds that would be expected to have similar properties, even if it could be established that there would be a reasonable expectation that the 8-hydroxy compound would in fact have similar properties.

It is also respectfully submitted that no reasonable expectation of success from making pharmaceutical compositions of the 8-hydroxy analogue of tofisopam was established by the data presented in Ito, assuming that "success" would be considered to be a compound with the desirable pharmacological properties of tofisopam but reduced toxicity. As discussed above, even if it were established that the 8-hydroxy compound should have similar properties to the 7-hydroxy compound, then all that could reasonably have been expected from the teachings of Ito would have been that the 8-hydroxy compound would have markedly inferior pharmacological properties compared to tofisopam. However, the data in Table 10 in fact show that the effect of replacing the methoxy groups in tofisopam with hydroxyl was highly unpredictable – ranging from compounds such as TF3, having reasonable activity across a range of assays, to TF5, having no activity in any pharmacological assay. It is noteworthy, moreover, that the only compound investigated by Ito which in fact had an 8-hydroxy group, as found in the compound of the presently claimed compositions was TF5, the compound Ito reports as lacking any pharmacological activity in the assays. There would have been no way to tell *a priori* whether the 8-hydroxy compound presently claimed would be more like TF2 (the 7-hydroxy compound), TF5 (the only compound in Ito with an 8-hydroxy group) or be completely different from any the other compounds. There could have been no reasonable expectation of success when the results were as unpredictable as is established by Ito.

With regard to the final requirement for establishing a *prima facie* case of obviousness, the references do not teach or suggest all the limitations of all the rejected claims. Specifically, the references fail to teach formulating the isolated (R) or (S) enantiomers, nor formulating the isolated (R) or (S) enantiomer having specific levels of enantiomeric purity as required by the dependent claims. The examiner's rationale as to why it is apparently acceptable effectively to overlook these limitations in the rejected claims

again appears to amount to applying a *per se* rule that if a racemate is obvious then the isolated (R) or (S) enantiomers are also obvious. This is evident from the fact that the examiner has done no more than state that the person skilled in the art would know that it would be possible to separate the (R) and (S) enantiomers would expect that they would have different properties. The examiner has not provided references or other evidence to support either assertion. While the applicants agree that it is often possible to separate (R) and (S) enantiomers, applicants disagree completely that the result of separating the (R) and (S) enantiomers would have been predictable.

The applicants first respectfully point out that the courts have soundly rejected the notion of *per se* rules of obviousness based on alleged structural similarity, and therefore, even if the racemic compound is obvious, the (R) and (S) enantiomers cannot be considered automatically *prima facie* obvious. *Ochai* explained that "The use of per se rules...flouts section 103 and the fundamental case law applying it ...[O]ur precedents do not establish any per se rules of obviousness". *In re Ochiai*, 71 F.3d. 1565, 1572 (Fed. Cir. 1995). Specifically with regard to the *per se* obviousness of an isolated enantiomer over the racemate, the Board of Patent Appeals and Interferences has held that a disclosure of a racemate "will rarely, if ever, suffice as substantial evidence of obviousness under § 103(a). ... [T]he examiner must explain why the differences would have been obvious, and the explanation must be supported by evidence in the record." *Ex parte Bonfils*, 64 USPQ2d 1456, 1461 (Bd. Pat. App. & Int. 2002). Further the Federal Circuit has mandated that *Graham* findings must be made "in *every* case to establish a prima facie case of obviousness." *Ortho McNeil Pharm., Inc. v. Mylan Labs., Inc.*, 348 F. Supp. 2d 713, 749 n.19 (citing *In re Mayne*, 104 F.3d 1339, 1341 (Fed. Cir. 1997) (emphasis in the original). Similarly, the Federal Circuit has clearly stated that "generalization should be avoided insofar as specific chemical structures are alleged to be prima facie obvious one from the other." *In re Grabiak*, 769 F.2d 729, 731 (Fed.Cir.1985).

The applicants also respectfully point out that the person skilled in the art was aware that the pharmacological properties of the isolated (R) and (S) enantiomers relative to each other and the racemate were not predictable *a priori*, as the summary of Islam, *et al.*, *Drug Safety*, **1997**, *17*(3), 149-165, provided herewith, and quoted below, clearly establishes:

> Interactions of both isomers may differ at the active sites through which pharmacological action is mediated. Actions and levels of activity of the stereoisomers in vivo may also differ. All the pharmacological activity may reside in a single enantiomer, whereas several possibilities exist for the other enantiomer-- it may be inactive, have a qualitatively different effect, an antagonistic effect or produce greater toxicity. Two isomers may have nearly identical qualitative pharmacological activity, qualitatively similar pharmacological activity but qu antitatively different potency, or qualitatively different pharmacological activity.

The only rationale provided by the examiner that it would have been obvious to prepare compositions of the (R) or (S) enantiomers with specified optical purity in view of Kórósi and Ito are the assertions that it would allegedly have been obvious to determine where in a disclosed set of percentage ranges is optimum, and that it would have been obvious to discover the optimum value of a result effective variable. It is not clear to the applicants, however, to what "*disclosed* set of percentage ranges" the examiner is referring here – none of the compounds in Kórósi or Ito appear to have been prepared optically pure, so there is no "disclosed set of percentage ranges". The examiner has also not cited any other reference making such a disclosure. There appears only to be the examiner's bare assertion that optimization of the optical purity would be obvious.

Similarly, although the examiner states that it is obvious to optimize a result effective variable, the examiner has not provided any evidence showing that the optical purity of the compounds in the compositions of the invention would have been recognized by the person skilled in the art as being result effective. The applicants respectfully remind the examiner that "[a] particular parameter must first be recognized as a result-effective variable, i.e., a variable which achieves a recognized result, before the determination of the optimum or workable ranges of said variable might be characterized as routine experimentation." MPEP 2144.05B (citing *In re Antonie*, 559 F.2d 618 (CCPA 1977)). Neither Kórósi nor Ito reveal that the optical purity of the compounds disclosed therein are in any way "result-effective", and the examiner has not cited further references or provided any additional evidence. Again, only a bare assertion by the examiner appears.

The examiner has therefore failed to establish a *prima facie* case of obviousness The examiner may not rely on *per se* rules of obviousness to establish the obviousness of the

presently claimed compositions regardless of whether the compound therein is isomeric with known compounds, or an isolated single enantiomer of allegedly obvious compounds, since *per se* rules of obviousness are not recognized under our patent law. Ito teaches away from the examiner's suggested modification of Ito and Kórósi, and, the data in Ito clearly establish that there would have been a lack of predictability and reasonable expectation of success from making the modifications which the examiner suggests would have been obvious.

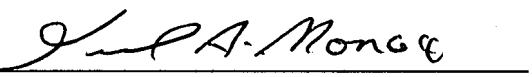
It is therefore respectfully submitted that the examiner's rejection of claims 1-12 under 35 U.S.C. § 103(a) over Kórósi in view of Ito should be withdrawn.

Conclusion

In view of the above remarks, all the examiner's rejections are believed to have been overcome, and that the application is now in condition for allowance. An early office action toward that end is therefore earnestly solicited.

Respectfully submitted,

Herbert W. Harris, *et al.*

BY 

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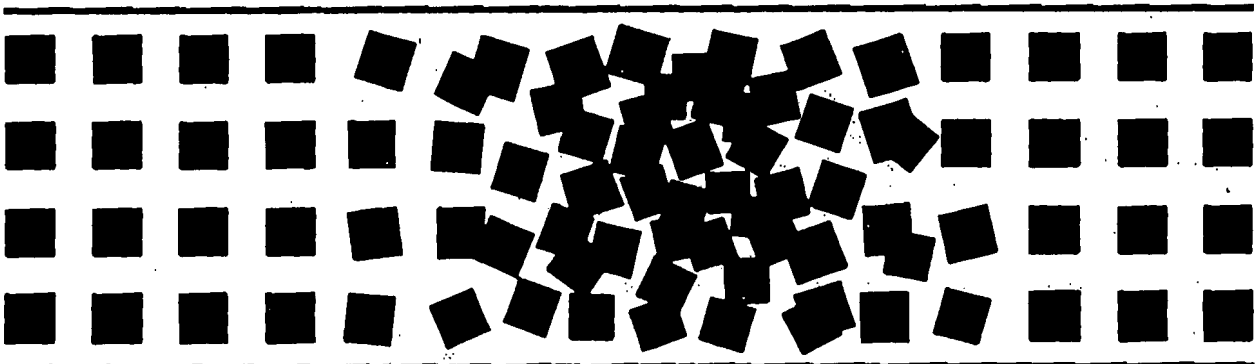

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therapy of human diseases of all kinds are natural products and that the microbial, plant, and marine worlds are a virtually inexhaustible source of biologically active novel compounds that provide important leads for subsequent structural modification. Also, with the introduction of high-speed computers capable of performing 100 million calculations per second and possessing sophisticated graphics capability, the possibility of designing compounds based on known characteristics of presumed targets has now become a realistic goal. These supercomputers can graphically illustrate the nature of chemicals capable of binding to specific receptors, and chemical synthesis can be simulated through computer programs developed to synthesize complex molecules based on known synthetic reactions. The future of the design of new anticancer drugs may lie in the capacity of these high-speed computers to offer compounds designed on a rational basis for later biologic testing.

Inherent in all screening systems is the tenet that biologic activity in some preclinical system must be demonstrated before human testing is performed. To date, no currently marketed, useful anticancer agent is devoid of such preclinical antitumor effect. Workers in cancer drug development often face advocates for various anticancer agents who, by reason of theory or personal interest, believe their material shows great promise as a human cancer treatment. Whether compounds selected for theoretical reasons, without demonstrated biologic activity in rodent systems, might be effective in human tumors has never been adequately tested. Without demonstrated activity in an in vitro system or in one of the many rodent systems available, the decision usually is not to initiate clinical testing of such materials. Given the need to use some selection criteria to narrow the choice of drugs for clinical trials, screening systems are likely to remain the mainstay for decision-making.

FORMULATION AND TOXICOLOGY TESTING

Formulation and production of anticancer drugs, required before anticancer drugs can proceed to toxicology studies and clinical trials, often present formidable obstacles for chemists. Anticancer agents with considerable activity in rodents have been discarded for lack of an adequate formulation for human use. This is particularly true of the more complicated products extracted from plants. Once these formulation problems have been solved, however, preclinical testing for toxicity is a requirement of development. Then, the Food and Drug Administration (FDA) will approve an investigational new drug application (INDA) that permits clinical testing.

Toxicology testing has evolved over the last decade from complicated testing in rodents, dogs, and monkeys to a less expensive and simpler system that relies on toxicity testing primarily in mice. Large amounts of data accumulated since the beginning of anticancer drug development have allowed comparisons to be made across species with respect to common toxicity of chemicals. These data have shown that there is no real safety advantage in using larger animal species instead of rodents. In the current system, implemented in 1980, the dose–response curve of a new drug is first developed in mice. The lethal dose (LD) in 10%, 50%, and 90% of animals is determined and the reproducible lethal dose in 10% of tested animals (LD_{10}) is used as the basis for establishing the initial dose in clinical trials. Usually, 10% of the LD_{10} dose in rodents is selected for the initial human dose; this dose is first tested for toxicity in dogs, prior to use in humans, to minimize the risks associated with administering an unknown compound to humans. Although correlation of toxic effects on rapidly dividing normal tissue among rodents, dogs, monkeys, and humans is good, correlation of other toxic effects is not as consistent.[149] Therefore routine pathologic examination of rodent tissue is not always performed prior to clinical testing.

TRANSLATION OF DOSES ACROSS SPECIES. All drugs should be given in reference to either body weight or surface area. The preferable reference point is body surface area, because better cross-species comparisons can be made and because doses calculated from body surface area allow doses to be determined for adults and children without further adjustment. The assumptions leading to the dose conversion factors have been described in detail by Freireich and coworkers[24] and are shown in Table 16-6, which is useful in converting doses in milligrams per kilogram to the comparable milligram per square meter dose. Table 16-7 shows the procedure for conversion of a milligram per kilogram dose in rodents, monkeys, or dogs to the equivalent dose in man.

TABLE 16-6. Representative Surface Area to Weight Ratios (km) of Various Species*

Species	*Body Weight (kg)*	*Surface Area*	*Surface Area to Weight Ratio (km)*
Mouse	0.02	0.0066	3.0
Rat	0.15	0.025	5.9
Monkey	3	0.24	12
Dog	8	0.40	20
Human			
Child	20	0.80	25
Adult	60	1.6	37

* To express a mg/kg dose in any given species as the equivalent mg/m² dose, multiply the dose by the appropriate *km*. In the adult human, for example, 100 mg/kg is equivalent to 100 mg/kg × 37 kg/m² = 3700 mg/m².

TABLE 16-7. Equivalent Surface Area Dosage Conversion Factors*

	Mouse, 20 g	*Rat, 150 g*	*Monkey, 3.0 kg*	*Dog, 8 kg*	*Man, 60 kg*
Mouse	1	½	¼	1/8	1/12
Rat	2	1	½	¼	1/7
Monkey	4	2	1	3/8	⅕
Dog	6	4	5/3	1	½
Man	12	7	3	2	1

* This table gives approximate factors for converting doses expressed in terms of mg/kg from one species to an equivalent *surface area* dose expressed in the same terms mg/kg in the other species. For example, given a dose of 50 mg/kg in the mouse, what is the appropriate dose in man assuming equivalency on the basis of mg/m^2?

$$50 \text{ mg/kg} \times 1/12 = 4.1 \text{ mg/kg}$$

EARLY CLINICAL TRIALS OF ANTITUMOR AGENTS

Antitumor agents go through four phases of clinical testing before they are accepted for general medical practice, marketed, or discarded (Fig. 16-6).[137,150-153] The average time from discovery of an effective antitumor agent to marketing of that agent is quite long, in the range of 10 to 12 years. To facilitate access to drugs for desperately ill cancer patients before the drugs are marketed, anticancer drugs with known efficacy are made available to physicians by the NCI in the premarketing phase (Tables 16-8 and 16-9).

Table 16-10 details the phases of clinical testing and the main purpose of each step. Phase I trials are done on small groups of patients, usually no more than 15 to 30 per study. Although the main purpose of Phase I trials is to identify a maximally tolerated dose (MTD) in one of several schedules suggested by the preclinical data, patients are entered into Phase I trials with therapeutic intent. For most of the effective anticancer drugs, some therapeutic effect was often seen even in Phase I trials. Because a limited number of patients with a variety of diseases are treated in Phase I trials, and doses may be below the ultimate therapeutic range in a fraction of the patients, the absence of any positive effect in a Phase I trial is not sufficient reason to discontinue testing of a drug. The only reason not to proceed to a Phase II study is prohibitive toxicity in Phase I trials. Escalation of doses in Phase I trials is usually done by a modified Fibonacci system.[150] Doses are first doubled and then increased at decreasing increments of 66%, 50%, and 33% in succeeding groups of patients (usually three at a time) until limiting toxicity is noted. Recently, attempts have been made to rationalize and accelerate dose escalation by the systematic use of preclinical pharmacologic data.[154] This approach has relied on the assumption that the elimination rate of a drug determines its C × T, and further assumes that for agents showing no major differences in target cell sensitivity, schedule dependence, or toxicity between mouse and man, the C × T at the mouse LD_{10} and the human MTD should be similar. These assumptions lead naturally to a simple algorithm for escalating doses by targeting the human C × T in a Phase I trial to the mouse C × T at LD_{10}.[155] The steps are as follows: (1) determine the mouse LD_{10} (part of the routine preclinical toxicology testing discussed earlier), (2) determine the

TABLE 16-8. National Cancer Institute Classification of New Anticancer Drugs in Clinical Testing

Group A Drugs
This group includes drugs in Phase I clinical trials and Phase II clinical trials in specified tumors. Protocol acceptance and drug distribution are limited to clinical investigators.

Group B Drugs
This group includes drugs already tested in initial Phase II studies and of clinical interest. Protocol acceptance and drug distribution are extended more broadly to clinical cooperative groups, NCI contractors, and cancer centers.

*Group C Drugs**
Group C includes drugs that demonstrate efficacy within a tumor type in more than one study, that alter the pattern of care of the disease in question, and that are administered safely by properly trained physicians without requiring specialized supportive care facilities. This group includes the following:

1. Azacytidine (NSC 102816)—for refractory acute myelogenous leukemia
2. Ervinia asparaginase (NSC 106977)—for acute lymphatic leukemia in patients sensitive to *E. coli* L-asparaginase
3. Hexamethylmelamine (NSC 13875)—for ovarian carcinoma
4. Amsacrine (NSE 249992)—for refractory myelogenous leukemia

* Drugs in Group C are available for use by physicians for specific indications.

TABLE 16-9. Procedure for Obtaining Drugs in Group C of the National Cancer Institute New Anticancer Drug Classification

A physician must be registered with the NCI as an investigator having completed an FDA-Form 1573.
A written request for the drug, indicating the disease to be treated, must be submitted.
Use of the drugs shall be limited to indications outlined in the guidelines that will be provided to the physician.
All adverse reactions must be reported to the Investigational Drug Branch, DCT, NCI.
Office of the Chief, Investigational Drug Branch, CTEP*, DCT, National Cancer Institute, 7910 Woodmont Avenue, Landow Building, Room 4A22, Bethesda, MD 20892 (301-496-6138).

* CTEP = Cancer Therapy Evaluation Program.

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METHODS FOR IDENTIFYING A DEFAULT CROSS-SPECIES SCALING FACTOR

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As a matter of necessity, the potential for a chemical agent to produce adverse health effects in humans is often investigated in experimental animals, typically rats and mice. The use of these surrogates is premised upon the high degree of physiological, biochemical, and anatomical similarity among mammalian species; toxicological effects observed in the experimental animals may be taken as evidence that humans might show similar responses to equivalent chemical exposures. What constitutes "equivalent" is of course the challenging aspect of this assumption and comprises the topic of this paper.

The importance attached to the concept of toxicologically equivalent doses varies somewhat depending on the stage of the risk assessment process. For qualitative evaluations such as hazard identification, there is a lesser need to ensure that doses are equivalent between humans and experimental animals. In fact, dosing animals at much higher levels than probable human exposures is the default approach, intended to elicit a detectable response in a limited population of experimental animals. When the aim is dose-response extrapolation and setting acceptable levels of human exposure, however, one must be able to specify a quantitative relationship between the dose levels in humans and in animals that is expected to result in the same degree of adverse effect. In this case, it is necessary to take into account the pronounced difference in scale between the tested model organisms and humans. Even if fundamental similarity is presumed, one must allow for the fact that the greater size and lifespan of the human relative to the experimental animal has a significant impact on the amount of chemical intake needed to provoke a given response.

The question of cross-species dose equivalency has traditionally been handled differently in assessment of cancer versus non-cancer toxicity. Although approaches within each realm have varied, the key difference between them is that cancer risk assessment has focused on explicit extrapolation from animal to human doses, using a quantitative adjustment to calculate a presumed "human equivalent" dose, whereas non-cancer assessment has relied on a semiquantitative allowance for uncertainty in the human dose that may be equivalently toxic, accomplished by applying an uncertainty factor that does not pretend to be a precise extrapolation. The dose extrapolation in cancer assessment is (at least by intention) a central

estimate, producing a single best guess at an equivalent human dose while acknowledging that there is a distribution of uncertainty around this extrapolation that rarely receives analytical examination. The "animal-to-human" uncertainty factor of non-cancer assessment, in contrast, is intended as a lower bound on the range of possible human doses that might be toxicologically equivalent (in view of chemical-to-chemical variation in how sensitive humans are vis-à-vis experimental animals), and as such ought to be somewhat conservative on average. A harmonized approach should seek to find commonality between these approaches. This would seem to entail bringing a focus on best-guess extrapolation of equitoxic doses to assessment of non-cancer endpoints and a more explicit allowance for uncertainty around dose-equivalence calculations to the assessment of cancer endpoints.

In the absence of chemical-specific information sufficient to do otherwise, the guidance of the U.S. Environmental Protection Agency (EPA) for carcinogen risk assessment is to apply a default animal-to-human oral dose extrapolation based on presumed toxicological equivalence of daily doses scaled by the ¾-power of body weight (i.e., $mg/kg^{3/4}$/day doses are presumed equivalent) (US EPA 1992, 1999). (Inhalation exposures are assessed according to methods developed for non-cancer assessment, as described in US EPA 1994; Jarabek 1995). When such doses are reexpressed in the traditional units of mg/kg/day, it is seen that a human dose about 7-fold less than a mouse dose or 4-fold less than a rat dose are being presumed to be equivalently carcinogenic.

The current default practice for non-cancer risk assessment (Dourson, 1996) is to apply an animal-to-human uncertainty factor (U_A), reducing the human mg/kg/day dose by 10-fold to allow for the fact that humans might be up to this much more sensitive than the test species to which they are being compared. The factor of 10 is based on a combination of professional judgment, experience, and empirical data suggesting that a larger adjustment is rarely needed (Dourson and Stara, 1983; Dourson et al., 1992, 1996). While this approach has worked well in practice, it has the shortcoming of failing to address the actual central estimate of the expected human equivalent dose. This has the following consequences:

- it is hard to tell how much of the 10-fold is needed adjustment to achieve human dose-equivalency on average versus an allowance for chemical-by-chemical uncertainty in that adjustment. Accordingly, it is hard to know how conservative the assessment is on average;

APPENDIX A

Mathematical Relationships For Scaling According To A Power of Body Weight

Allometric relationships are premised upon the following equation:

$$Y = a \cdot BW^b,$$

where *Y* is the feature (an organ weight, blood flow, metabolic rate, *etc.*), *BW* is body weight, and *b* is a power of weight, and *a* is a proportionality constant.

In the special case where the value of *b* is 1.0, the plot is a straight line and is described as isometry. Organ volumes tend to scale in a direct relationship with body weight and so can be described as isometric. When *b* is a value different than 1.0 (*e.g.*, 0.75), the relationship between *Y* and *BW* is not a straight line and is described as allometric. Many metabolically-related parameters and in general all physiological rate processes (*e.g.*, heart beats, cardiac output, glomerular filtration rate, minute volumes, oxygen consumption) tend to be described by allometric equations.

When the scale of values considered is extremely large (*e.g.*, the body weight range between elephants and mice), the expression can conveniently be expressed in logarithmic form:

$$\log Y = \log (a \cdot BW^b) = \log a + b \cdot \log BW.$$

That is, on a log-log plot, the relationship is a line with slope *b*.

Some parameters have only time as a determinant, such as metabolic half-lives (fraction per hour) or respiration (breaths per hour). Other parameters of interest, such as cardiac output (ml/hr) are more conveniently related in proportion to overall body size. This is accomplished by dividing out the body mass of the organism and then examining the scaling of amount per kilogram. This yields value having units with (mass*time) in the denominator. Thus:

$$Y/BW = (a \cdot BW^b)/M$$

Given that the exponent for body mass is 1.0, if the value of $b = 0.75$, then

$$Y/BW^{1.0} = (a \cdot BW^{0.75})/BW^{1.0}$$

or

$$Y' = a \cdot BW^{-0.25}$$

where *Y'* is the value of interest expressed in a body mass specific manner (liters of blood per hour).

If it is presumed that daily doses are equally toxic in differently sized species when they are proportional to body weight to the power *b*, this means that the raw daily amounts *A* (in mg) can be normalized by dividing by BW^b, *i.e.*,

$$A_1/BW_1^b = A_2/BW_2^b,$$

where 1 and 2 refer to two species. For example, if species 1 is a 35 g mouse and species 2 is a 70 kg human, and if b=3/4, then when A_1 is a 1 mg dose to a mouse, this constitutes 1 mg/(.035kg)$^{3/4}$ = 12.4 mg/kg$^{3/4}$. A toxicologically equivalent dose A_2 to a human would also equal 12.4 mg/kg$^{3/4}$, that is

$$A_2/70^{3/4} = 12.4$$

$$A_2 = 12.4 \cdot 70^{3/4} = 299 \text{ mg.}$$

The total dose is almost 300-fold bigger, which is less than proportional to the body size difference of 2000-fold (= 70/0.035).

If these two mg doses A_1 and A_2 are expressed as mg/kg/day doses D_1 and D_2, then they are

$$D_1 = A_1/BW_1 = 1/.035 = 28.6 \text{ mg/kg/day}$$

$$D_2 = A_2/BW_2 = 299/70 = 4.27 \text{ mg/kg/day.}$$

That is, the human dose is 28.6/4.27 = 6.7-fold lower on a body-weight basis.

If one starts with a mouse dose *already* scaled to body weight (*i.e.*, D_1 in mg/kg/day) and wishes to scale this (using body weight to the ¾ power scaling) to a human mg/kg/day dose D_2, then the "scaling factor" is 6.7 since $D_2 = D_1/6.7$. The size of this factor is given by $(BW_2/BW_1)^{1/4}$, in our case $(70/.035)^{1/4}$ = 6.7. The reason this is so (and the ¼ power is used) is because (using relationships shown above)

$$A_1/BW_1^{3/4} = A_2/BW_2^{3/4}$$

Since $1/BW^{3/4} = BW^{1/4}/BW$, this can be rewritten as

$$(A_1/BW_1) \cdot BW_1^{1/4} = (A_2/BW_2) \cdot BW_2^{1/4}$$

$$D_1 \cdot BW_1^{1/4} = D_2 \cdot BW_2^{1/4}$$

$$D_2 = D_1 \cdot (BW_1^{1/4}/BW_2^{1/4}) = D_1/(BW_2/BW_1)^{1/4}$$

Figure 1A. Plasma toxicant concentrations with human and mouse doses scaled according to bodyweight. Humans and mice receive the same mg/kg body weight dose at the same dose intervals. The faster metabolism in the mouse leads to a more rapid attainment of steady state conditions and a lower cumulative body burden.

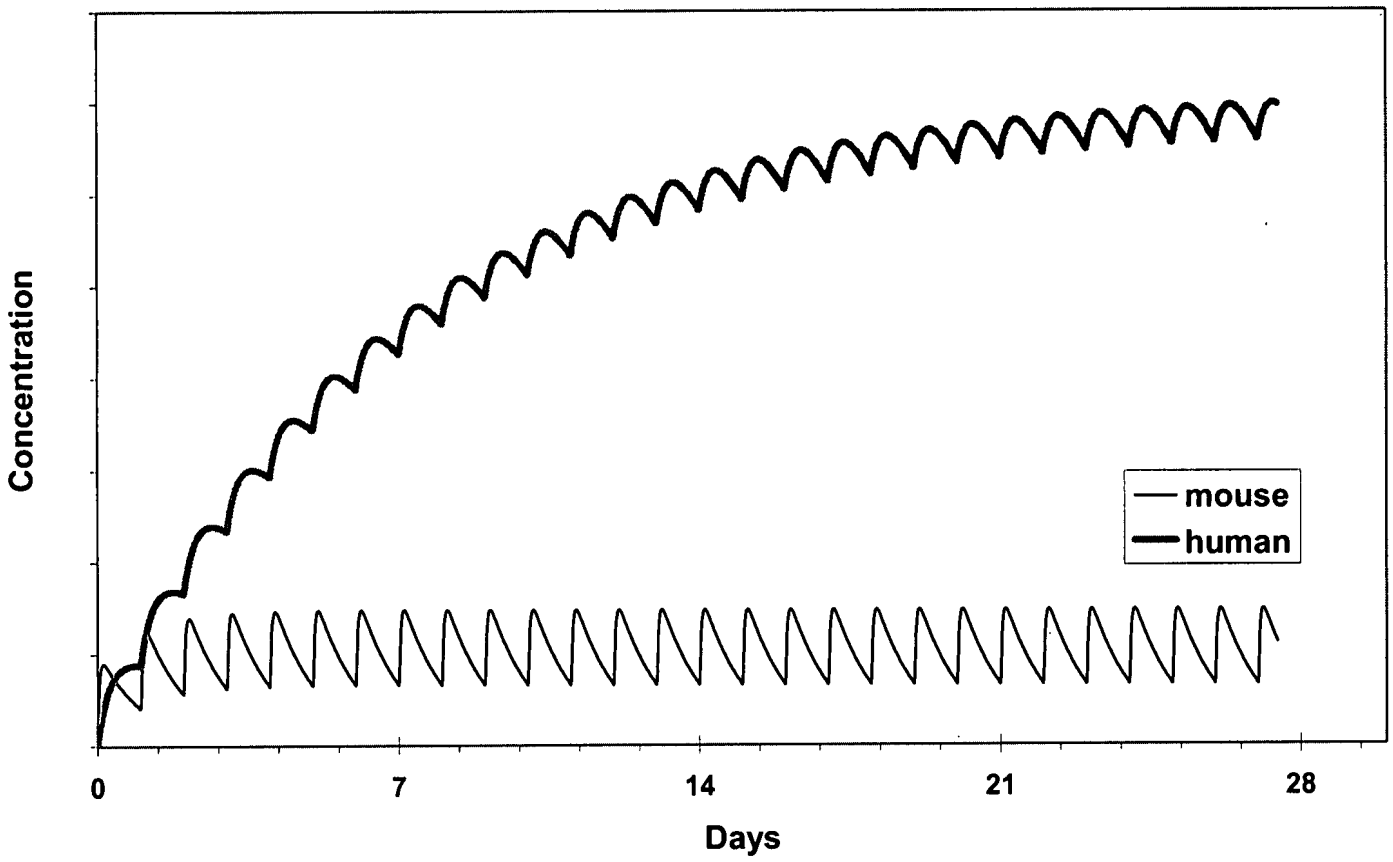


Figure 1B. Plasma toxicant concentrations with human and mouse doses scaled according to the ¾ power of bodyweight. This method of dose scaling leads to the same average steady state concentration and more similar AUCs compared to scaling to $BW^{1.0}$, as shown in Figure 1A.

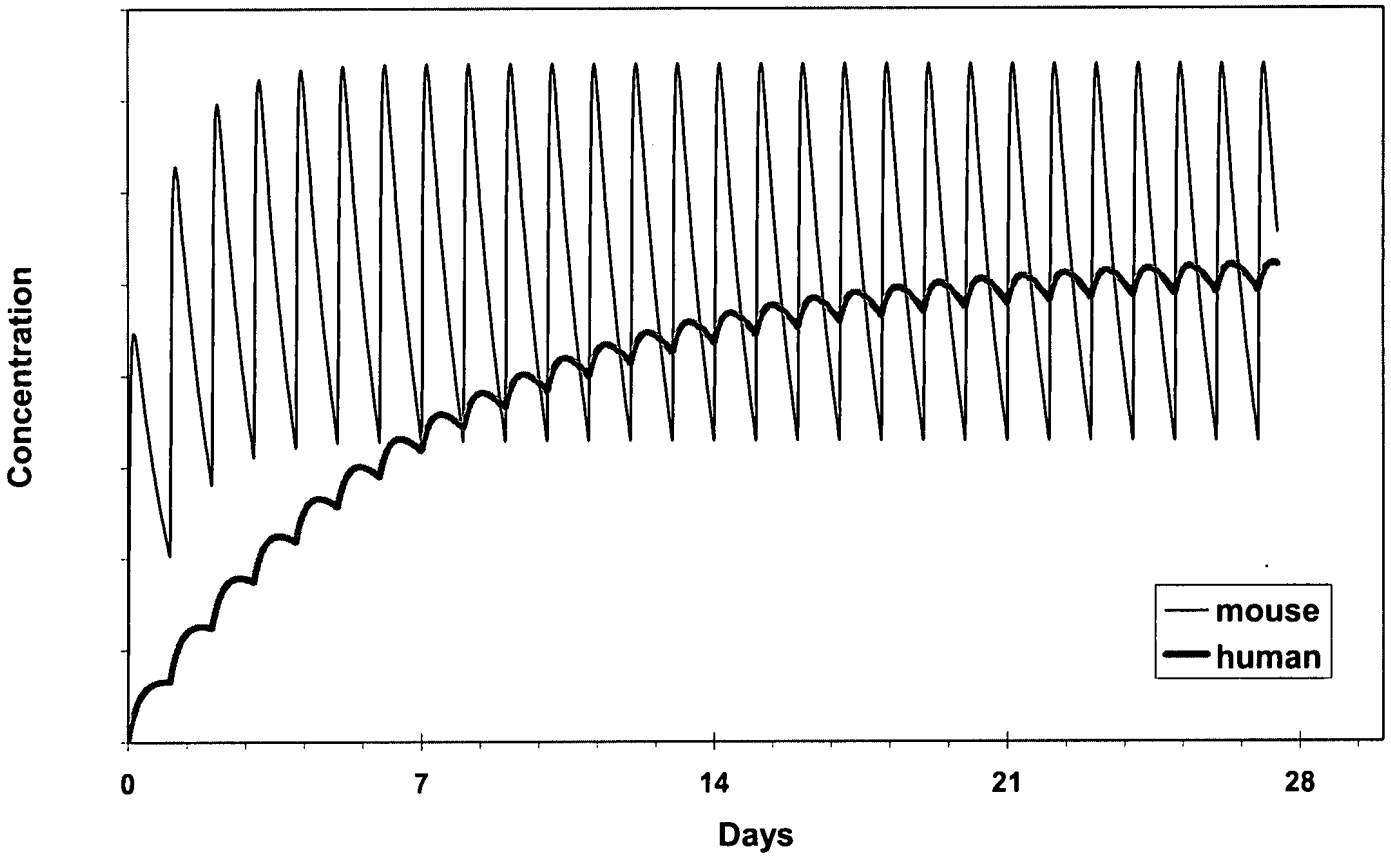


Figure 1C. Plasma toxicant concentrations with the dose interval adjusted according to scaling by the ¾ power of body weight. Plasma concentrations with daily doses, scaled according to $BW^{1.0}$, mice dosed daily and humans dosed every 7 Days. Adjusting the dose interval rather than the dose itself by multiplying the human dosing interval by the body weight difference between the two species raised to the −1/4 power (*i.e.*, $(0.035/70)^{-0.25} = 6.7$), also leads to the same average steady state concentration and similar AUCs.

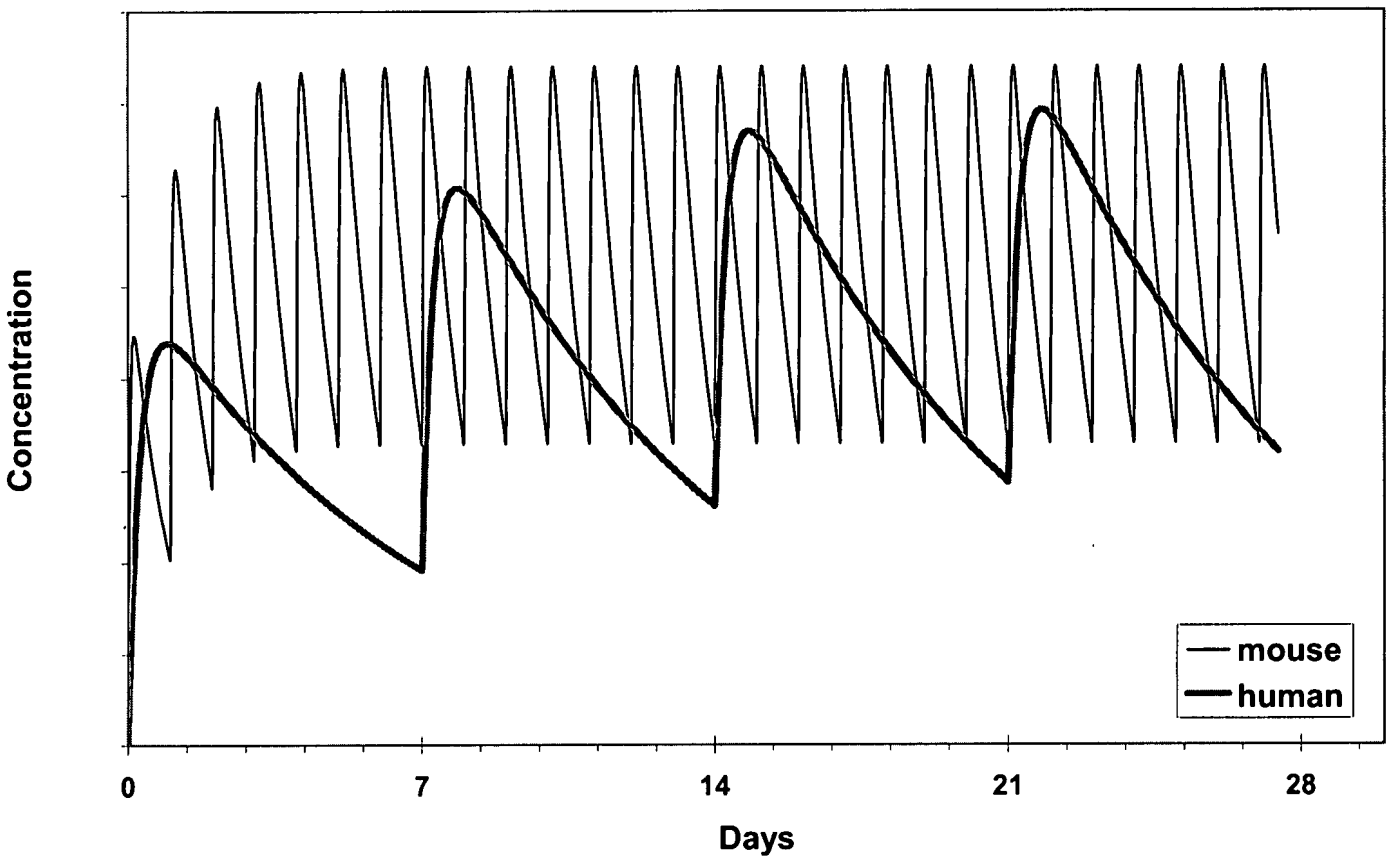
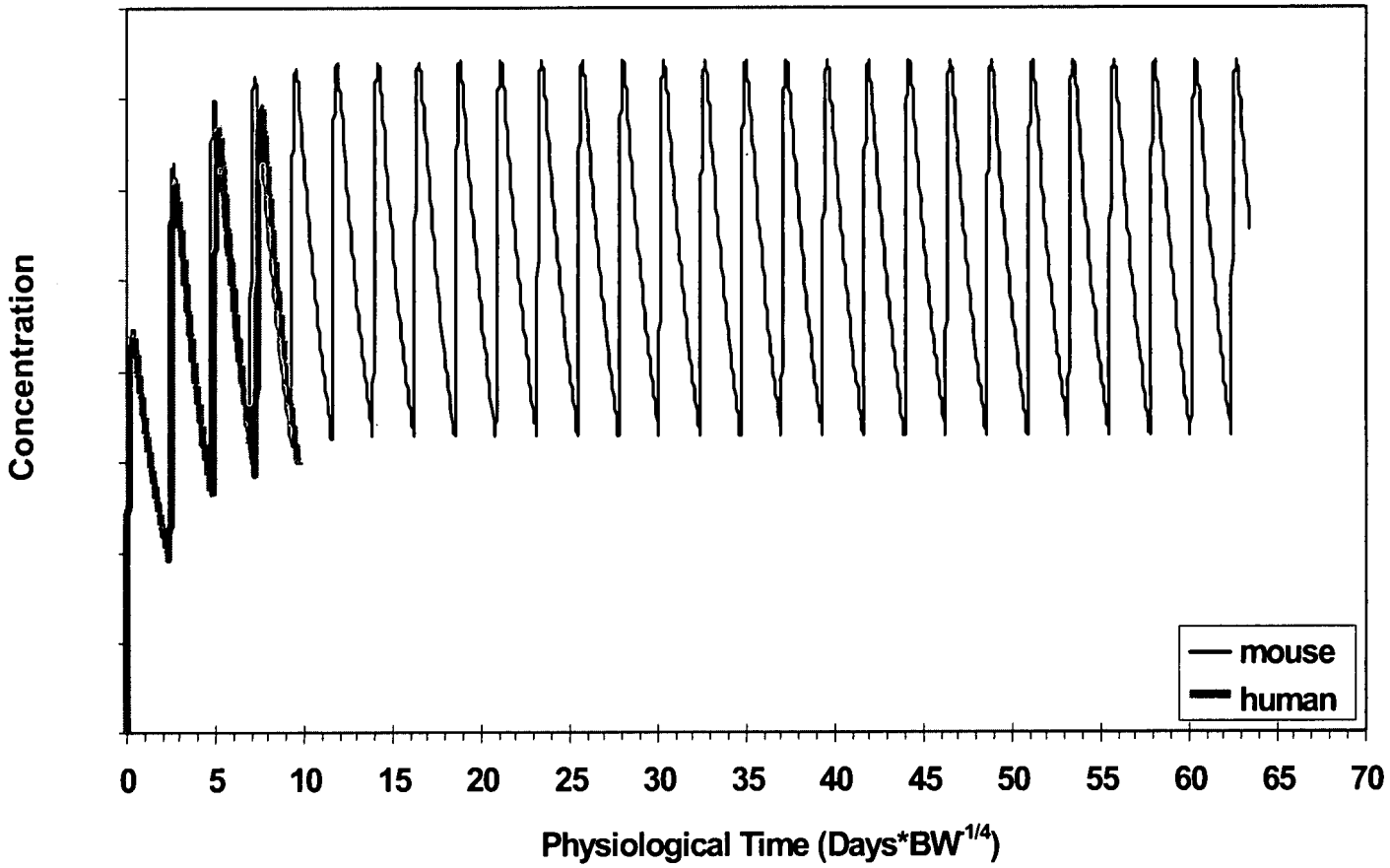


Figure 1D. Plasma toxicant concentrations scaled according to physiological time. Data from Figure 1C shown on an x-axis of physiological time. The x-values for the human and mouse data have been converted from chronological time to physiological time by multiplying the human x-axis data by the body weight difference between the two species raised to the −1/4 power (*i.e.*, $(0.035/70)^{-0.25} = 6.7$). This leads to overlapping of the human and mouse data.



COMMENTARY

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Pharmacological Importance of Stereochemical Resolution of Enantiomeric Drugs

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Summary

Drug enantiomers have identical properties in an achiral environment, but should be considered as different chemical compounds. This is because they often differ considerably in potency, pharmacological activity and pharmacokinetic profile, since the molecules with which they interact in biological systems are also optically active. Within biological systems, the metabolism of one isomer may be via a different pathway or occur at a different rate from that of the other isomer. Preferential binding of one isomer to plasma proteins may cause differences in circulating free drug and hence alter concentrations at active sites. Interactions of both isomers may differ at the active sites through which pharmacological action is mediated. Actions and levels of activity of the stereoisomers *in vivo* may also differ. All the pharmacological activity may reside in a single enantiomer, whereas several possibilities exist for the other enantiomer – it may be inactive, have a qualitatively different effect, an antagonistic effect or produce greater toxicity. Two isomers may have nearly identical qualitative pharmacological activity, qualitatively similar pharmacological activity but quantitatively different potency, or qualitatively different pharmacological activity.

To avoid adverse effects and optimise the therapeutic value of enantiomeric drugs, it is necessary that methods for the resolution of racemates be evolved and devolved to determine isomeric purity, establish the effectiveness of isomers of the drug, and detect the presence of an enantiomer with lower therapeutic activity and undesirable adverse effects. Even if a drug is given as a pure enantiomer, methods to discriminate between enantiomers are required because racemisation can occur both *in vitro* and *in vivo*. Methods developed for resolution of drug enantiomers should facilitate routine testing of single isomers and their metabolites, studies of pharmacological, toxicological and clinical effectiveness, routine analysis of racemates, pure enantiomers or intermediates in manufacturing processes, and investigation of the potential for inversion of an enantiopure drug substance during the early stages of drug development and therapeutic drug monitoring.

Molecular chirality is a fundamental phenomenon that plays an important role in biological processes. A wide range of biological and physical functions are generated through precise molecular recognition because enzymes, receptors and other natural binding sites within biological systems interact with different enantiomers in decisively different ways. Consequently, pharmaceutical companies and drug regulatory bodies have become aware of the differential efficacy and tolerability of enantiomers of racemic drugs.[1-3] This has lead some drug companies to develop drugs that comprise single enantiomers.[4-6] Enantiomeric drugs have become increasingly important over the last 20 to 30 years, since about 56% of drugs currently in use are chiral compounds and 88% of these chiral synthetic drugs are used therapeutically as racemates.[7,8] The purpose of this review is to highlight the problems of using racemic drugs and to note whether these can be minimised by using pure enantiomers.

1. Pharmacological Importance of Enantiomeric Drugs

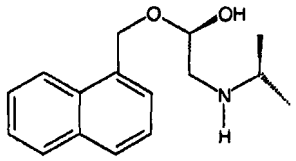
Stereoisomeric discrimination is remarkable in biological systems[9,10] where it is responsible for differences in physiological responses to the individual enantiomers of a given substrate and to the racemate as compared with the corresponding pure enantiomers.[11-13] The stereoselectivity of a biological system results in between-enantiomer differences in affinity for the active site of receptor systems and enzymes. The degree of stereoselectivity increases with the number of interaction points of the drug with the active site. Drug actions requiring more than 2 points of interaction will have a higher degree of selectivity than a 2-point interaction.[14-16]

It has become clear that enantiomeric drugs may differ in their pharmacodynamics[17-19] and pharmacokinetics[20-22] and both are important in describing the clinical pharmacology of chiral drugs.[15] The following examples highlight how different enantiomers of the same compound may have widely differing physiological activity.

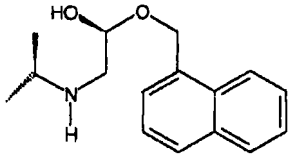
The stereoselectivity of the activity of propranolol (fig. 1) has been investigated for several pharmacological effects and diseases. Racemic propranolol and *R*-(+)-propranolol shift the haemoglobin-oxygen dissociation curve to the right *in vitro*.[23] Similar results have been noted in patients being treated with usual doses of racemic propranolol.[24] In hypertensive patients, the same daily doses of racemic propranolol and the non-β-blocking *R*-(+) isomer inhibited thrombin and arachidonic acid–induced platelet aggregation and thromboxane synthesis.[25] However, only the β-blocking *S*-(–) enantiomer is effective in treating patients with angina.[26]

Labetalol (fig. 2) is a diastereoisomer commercially available as equal proportions of 4 stereoisomers. Non-specific β_1- and β_2-blocking activity is predominantly conferred by the *R,R* isomer, while α_1-blocking activity is produced by the *S,R* isomer.[27] The other 2 isomers *S,S* and *R,S* probably contribute to drug activity, but to a much lower extent.

Tocainide (fig. 3) is a new antiarrhythmic racemic drug. In mice, the *R*-(–) enantiomer is 3 times more potent than *S*-(+) antipode as an antiarrhythmic drug.[28] Tocainide is an example of a recently approved racemic drug for which negligible information about enantiomeric activity is available.



S-(–)-Propranolol



R-(+)-Propranolol

Fig. 1. The absolute configuration of *S*-(–)-propranolol and *R*-(+)-propranolol.

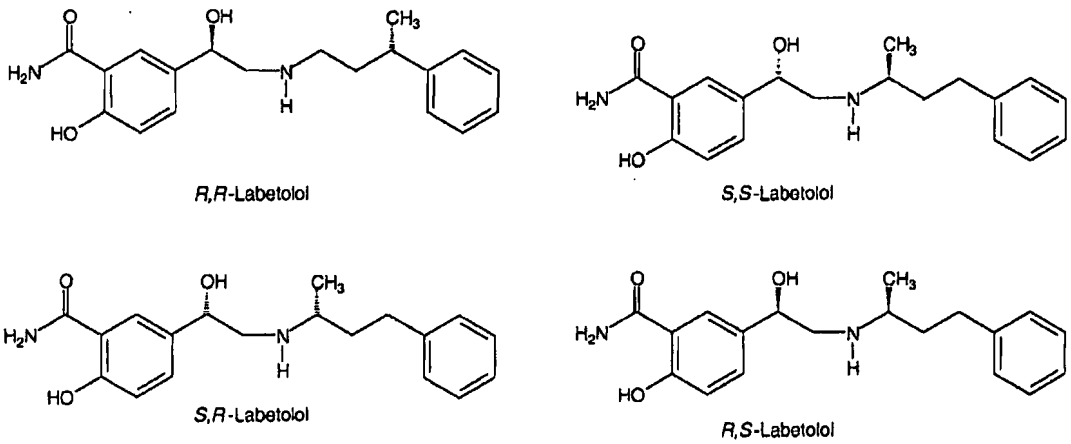


Fig. 2. The absolute configuration of *R,R*-labetalol, *S,R*-labetalol, *S,S*-labetalol and *R,S*-labetalol.

Ketamine (fig. 4) is an intravenous anaesthetic and analgesic agent. Clinically used ketamine is a racemic mixture of 2 isomers, *S*-(+)- and *R*-(–)-ketamine. Both the racemic mixture and the individual enantiomers have almost identical pharmacokinetic properties, but different pharmacodynamic effects. The *S*-(+) isomer of ketamine has about twice the anaesthetic and analgesic potency of racemic ketamine.[29] The *R*-(–) enantiomer, however, produced more psychic emergence reactions (37 versus 5% of patients) and more instances of postoperative agitation (10 versus 0% of patients) than the *S*-(+) enantiomer. Overall levels of postoperative anxiety were decreased by *S*-(+)-ketamine. Because differences in anaesthetic potency of the enantiomers led to the use of different doses, absolute levels of metabolites were higher in the *R*-(–)-ketamine group, and the possibility exists that differences in CNS toxicity are related to the activity of metabolites.[30]

A more dramatic example is the separation of convulsant and anaesthetic activities between the isomers of 5-(1,3-dimethylbutyl)-5-ethyl barbituric acid (DMBB) [fig. 5] and *N*-methyl-5-propyl barbiturate (MPPB) [fig. 6]. The *S*-(+) enantiomers are pure convulsants while the *R*-(–) enantiomers are anaesthetics.[31,32] The (+) enantiomers of pentobarbital (pentobarbitone) and other barbiturates, having the chiral centre in the side chain, produce more excitation than the (–) isomers,[33] but in no other case is the separation as striking as for DMBB. The *S*-(+)-DMBB isomer is convulsant and considerably more toxic in mice [dose lethal to 50% of animals (LD_{50}) = 3.1 mg/kg], than *R*-(–)-DMBB (LD_{50} = 72 mg/kg.[31]

2. Pharmacodynamics of Enantiomeric Drugs

Pharmacodynamics refers to what the drug does to the body, a measure of the time course of therapeutic or toxic responses to the presence of a drug. Many toxic reactions to drugs are an extension of

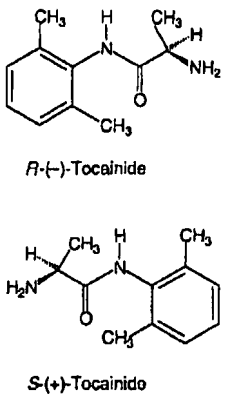


Fig. 3. The absolute configuration of *R*-(–)-tocainide and *S*-(+)-tocainide.

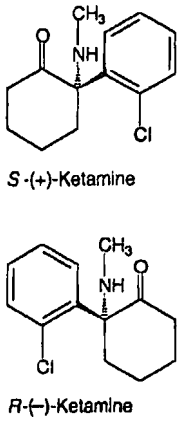


Fig. 4. The absolute configuration of *S*-(+)-ketamine and *R*-(–)-ketamine.

the mechanism responsible for the therapeutic effect. For example, reports show that accumulation of the 4-hydroxy-glutethimide metabolite of *R*-(+)-glutethimide plays an important role in acute glutethimide poisoning and in the incidence of other toxic effects of the drug.[34-38] It is generally agreed that enantiomers have different pharmacological properties. There are 4 possible reasons for different relative effects of enantiomeric drugs *in vivo*.

First, all the pharmacological activity may reside in one enantiomer, in which case the other enantiomer may be regarded as an impurity. The impurity may be inactive or have desirable or undesirable activity.[39]

3,4-Dihydroxyphenyl alanine (DOPA, fig. 7) in the body is present as the *S*-(–) enantiomer. The drug, called levodopa, is used to treat Parkinson's disease. The *R*-(+) enantiomer is responsible for potentially serious adverse reactions such as agranulocytosis.[40]

The antiarthritic activity of U-50488H is due entirely to the (–) enantiomer.[41] Similarly, only *S*-(+)-flurbiprofen (fig. 8) inhibits prostaglandin biosynthesis *in vitro* at therapeutic concentrations.[42] Furthermore, the pharmacological effects of medetomidine (fig. 9) lie solely with *S*-(+) isomer and the *R*-(–) isomer is essentially inactive.[43]

Secondly, 2 isomers may have nearly identical qualitative and quantitative pharmacological activity.[44] The enantiomers of promethazine (fig. 10), for example, have nearly identical antihistaminic properties and toxicity.[45]

Thirdly, enantiomers may have activity that is qualitatively similar but quantitatively different. Most drugs that exist as stereoisomers are in this category, e.g. warfarin,[46,47] tocainide,[28] verapamil,[48,49] propranolol,[50] thyroxine[51] and stiripentol.[52] If the drug has several pharmacological effects, greater selectivity of action may be achieved through the use of the enantiomer that has the greatest potency in terms of the desired activity. *S*-(–)-verapamil (fig. 11) might be chosen to treat supraventricular tachycardia, whereas *R*-(+)-verapamil (which increases coronary blood flow while producing less negative chromotropic and dromotropic activity) might be used to treat angina.[48]

Fourthly, enantiomers may have qualitatively different pharmacological activity. The desirable anaesthetic properties of ketamine reside primarily in the *S*-(+) enantiomer,[53,54] which is about twice as active as the racemate, and the undesirable excitatory and psychic disturbances are produced by the *R*-(–) enantiomer.[55,56] With the mixed adrenoceptor blocker labetalol, the α-blocking effect is in the *S,R* isomer, while β-blocking activities are in the *R,R* isomer.[27,57] The *S*-(–) enantiomer of ter-

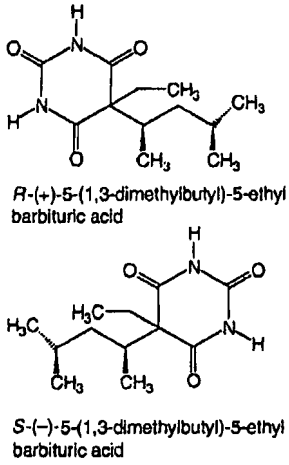
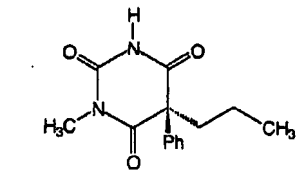
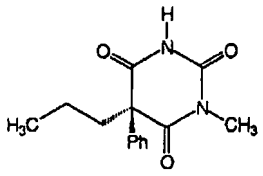


Fig. 5. The absolute configuration of *R*-(+)-5-(1,3-dimethylbutyl)-5-ethyl barbituric acid (DMBB) and *S*-(–)-DMBB.



R-(–)-*N*-methyl-5-propyl barbiturate



S-(+)-*N*-methyl-5-propyl barbiturate

Fig. 6. The absolute configuration of *R*-(–)-*N*-methyl-5-propyl barbiturate (MPPB) and *S*-(+)-MPPB.

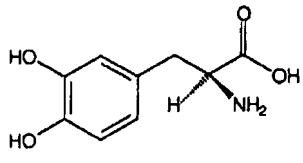
odiline (fig. 12) can be used to treat detrusor instability, whereas *R*-(+)-terodiline appears to induce ventricular arrhythmia.[58] In some cases, the pharmacological properties of a pair of enantiomers are marketed for different indications. (2*S*,3*R*)-(+)-Propoxyphene (fig. 13) is used as an analgesic, while its enantiomer (2*R*,3*S*)-(–)-propoxyphene is used as an antitussive.[20]

In summary, consideration of the stereoselective properties of enantiomers of chiral drugs may suggest therapeutic advantages over the use of racemates.

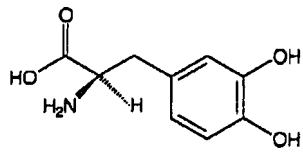
3. Pharmacokinetics of Enantiomeric Drugs

Pharmacokinetics refer to how the body affects the drug and how the body handles absorption, distribution and elimination of drugs in relation to their corresponding pharmacological, therapeutic or toxic response.[59] These physiological processes tend to be stereoselective; thus, enantiomers may vary in potency,[60,61] pharmacokinetic profile,[20] plasma disposition and urinary excretion.[19] Chiral drugs may have different pharmacokinetic actions because the metabolism of one enantiomer is different from or occurs at a different rate than that of the other.[62] There may also be preferential binding of one isomer to plasma proteins, causing differences in concentrations of circulating unbound drug and of drugs at active sites. Two enantiomers may also interact differently at receptors mediating pharmacological action. If the main therapeutic benefit exists in only one enantiomer, the other enantiomer may be inactive, have a qualitatively different effect, an antagonistic effect or greater toxicity.[40] The following examples have been selected to illustrate the pharmacokinetics of some enantiomeric drugs.

Salbutamol (fig. 14) is a long acting β_2-adrenoceptor agonist used orally or by inhalation to treat lung obstructive disease. The metabolism of salbutamol by sulphoconjugation is highly stereoselective, favouring the pharmacological active *R*-(–) enantiomer in human lungs,[63] bronchial epithelial

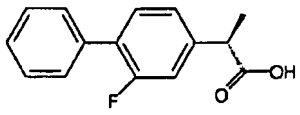


S-3,4-dihydroxyphenylalanine

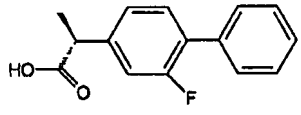


R-3,4-dihydroxyphenylalanine

Fig. 7. The absolute configuration of *S*-3,4-dihydroxyphenylalanine and *R*-3,4-dihydroxyphenyl-alanine.

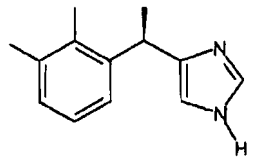


S-(+)-Flurbiprofen

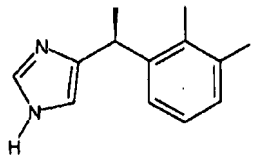


R-(–)-Flurbiprofen

Fig. 8. The absolute configuration of *S*-(+)-flurbiprofen and *R*-(–)-flurbiprofen.



R-(–)-Medetomidine



S-(+)-Medetomidine

Fig. 9. The absolute configuration of *R*-(–)-medetomidine and *S*-(+)-medetomidine.

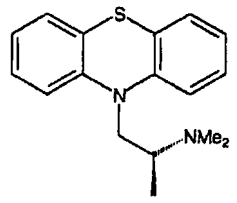
cells,[64,65] jejunal mucosal tissue and the liver.[66] The active *R*-(–) enantiomer undergoes significantly faster metabolism than the inactive *S*-(+) enantiomer, resulting in considerably lower bioavailability of the active enantiomer after oral administration.[67] The intrinsic clearance of *R*-(–)-salbutamol is about 10 times higher than that of the *S*-(+) enantiomer.[66] Although the drug is usually administered as a racemate, the inactive *S*-(+) enantiomer may have undesirable actions on lung function.[65]

Timolol (fig. 15) is a β-blocker effective in the treatment of hypertension, angina and glaucoma. Systemic absorption occurs when it is used topically to lower intraocular pressure and this may precipitate asthma or cardiac failure in some patients. *R*-Timolol has a much less potent effect on the β-receptor than *S*-timolol.[68] However, *R*-timolol is very effective in lowering intraocular pressure[68] and could be used to treat glaucoma without the risk of β-blocker adverse effects.

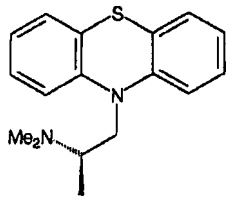
The stereospecificity of the pharmacokinetics of mefloquine (fig. 16) in children aged 6 to 24 months has been investigated using a coupled achiral-chiral chromatographic system. Maximum plasma concentrations, areas under the plasma concentration-time curve and apparent plasma elimination half-lives were higher for the (–) enantiomer than its antipode. In contrast, the apparent volume of distribution and total clearance values were higher for the (+) enantiomer.[69]

3.1 Clearance of Enantiomeric Drugs

Drugs are eliminated either by chemical biotransformation or by physical excretion from the body or a combination of the two. Clearance is the most important pharmacokinetic parameter for the description of the elimination of the drug. The

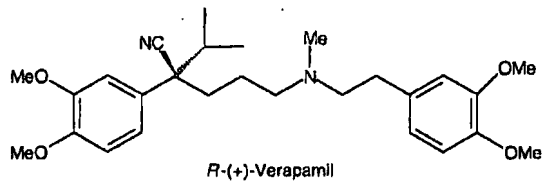


S-Promethazine

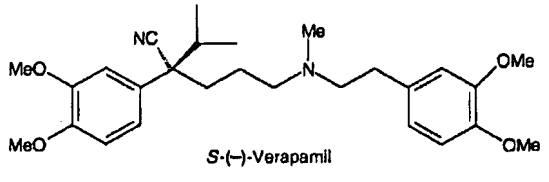


R-Promethazine

Fig. 10. The absolute configuration of *R*-promethazine and *S*-promethazine.

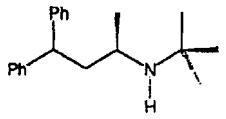


R-(+)-Verapamil

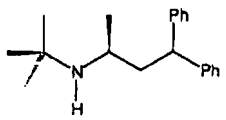


S-(–)-Verapamil

Fig. 11. The absolute configuration of *R*-(+)-verapamil and S-(–)-verapamil.



R-Terodiline



S-Terodiline

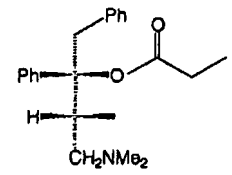
Fig. 12. The absolute configuration of *R*-terodiline and *S*-terodiline.

stereoselective clearance of enantiomeric drugs usually takes place by 3 different main pathways: oxidation, reduction and hydrolysis. The oxidation of propranolol, for example, exhibits different rates of transformation of the enantiomers. The formation of *R*-4-hydroxy-propranolol was 3 times faster than formation of the corresponding *S* enantiomer.[70] The ratio between formation of the more rapidly cleared enantiomer and of the less rapidly cleared enantiomer varied from 1.2 for phenprocoumon enantiomers (fig. 17) to 14.0 for acenocoumarol enantiomers (fig. 18).[71]

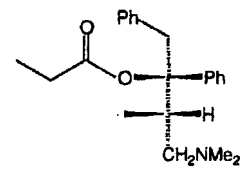
The analgesically active *R*-methadone (fig. 19) has a significantly longer mean elimination half-life than the optical antipode *S*-methadone (37.5 and 28.6 hours, respectively). The mean total volume of distribution is 496.6L for *R*-methadone and 289.1L for *S*-methadone. A significant difference is seen in the mean clearance between *R*- and *S*-methadone (0.158 and 0.129 L/min, respectively). However, the lagtime after oral administration and the bioavailability do not differ between the isomers.[72] Table I shows other examples of enantiomeric drugs which differ in their clearance rates.

Renal tubular secretion can also differ between drug stereoisomers. Quinidine and quinine (fig. 20) are stereoisomers differing in the configuration about 2 asymmetric carbon atoms. Calculating the clearance of unbound drug in serum revealed that the clearance of quinidine was 6.1 ± 2.3 times that of creatinine, whereas that for quinine was 1.5 ± 0.6 times that of creatinine.[78] There is stereoselective net renal tubular secretion of quinidine over quinine, indicating marked stereoselectivity of this renal tubular transport process.

Enantiomers of many drugs such as verapamil,[79] propranolol[80] and tocainide[81,82] have different pharmacological properties in humans, and there seems to be substantial interindividual variation in the ratios of the plasma concentrations of

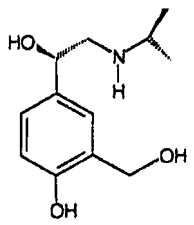


(2*S*, 3*R*)-Propoxyphene

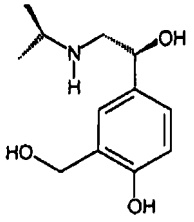


(2*S*, 3*R*)-Propoxyphene

Fig. 13. The absolute configuration of *2S,3R*-propoxyphene and *2S,3R*-propoxyphene.

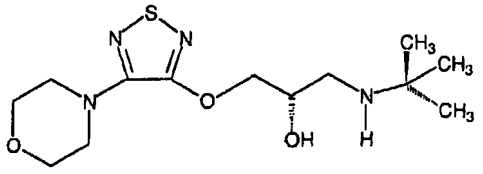


R-Salbutamol

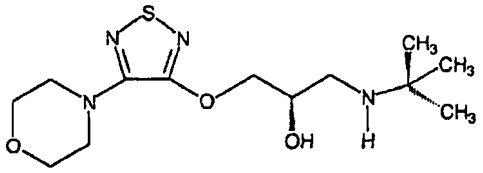


S-Salbutamol

Fig. 14. The absolute configuration of *R*-salbutamol and *S*-salbutamol.



R-(+)-Timolol



S-(–)-Timolol

Fig. 15. The absolute configuration of *R*-(+)-timolol and *S*-(–)-timolol.

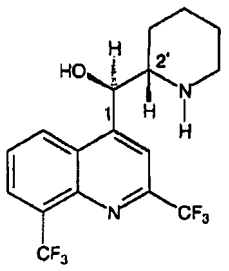
the drug enantiomers.[83] In the case of the anticoagulant drug acenocoumarol, the *R*-(+) enantiomer is cleared ≈15 times more rapidly than the *S*-(–) enantiomer, essentially due to differences in intrinsic hepatic clearance.[84] In general, the stereoselective metabolic clearance of different drug enantiomers may influence the therapeutic response of drugs.

3.2 Stereoselective Binding of Enantiomeric Drugs

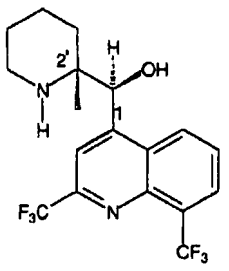
Drug enantiomers may bind to human plasma proteins stereoselectively.[85,86] For most drugs, human serum albumin has 2 high-affinity binding sites, the warfarin site (site I) and the benzodiazepine and indole site (site II).[41] Drugs that bind to site II do so in a highly stereoselective manner. The essential amino acid (–)-tryptophan binds to this site with an affinity about 100 times greater than that of (+)-tryptophan.[87] The (+) enantiomer of oxazepam exhibits 90% binding to human serum albumin compared to only 45% for levorotatory oxazepam.[88]

Binding of acidic chiral drugs, whether to site I on albumin or to human plasma proteins, is also uniformly stereoselective. For example, *R*-(+)-phenprocoumon binds to human serum albumin slightly less than the *S*-(–) enantiomer (91 versus 94%).[89] In contrast, binding of basic chiral drugs, whether to α_1-acid glycoprotein or to human plasma proteins, is relatively nonstereoselective. For example, both enantiomers of ketamine bind 55% to α_1-acid glycoprotein,[90] while (–)-propranolol binds to α_1-acid glycoprotein to a slightly greater extent than (+)-propranolol (87 *vs* 84%).[91] This is because ketamine is basic and propranolol is acidic.

In addition, different stereoisomers may have different binding parameters depending on the concentrations of the substances to which they bind. Etodolac (fig. 21), a nonsteroidal anti-inflammatory drug, is strongly bound to human serum albumin at both sites I and II. *R*-Etodolac is more extensively bound to the protein than the *S* enantiomer.[92,93] The stereoselective binding depends on the human serum albumin level, with the *R* enantiomer preferentially bound at 14.5 mmol/L and the stereoselectivity of etodolac binding being reversed at 580 mmol/L.[31,94]



1*R*,2'*S*-Mefloquine



1*R*,2'*R*-Mefloquine

Fig. 16. The absolute configuration of 1*R*,2'*S*-mefloquine and 1*S*,2'*R*-mefloquine.

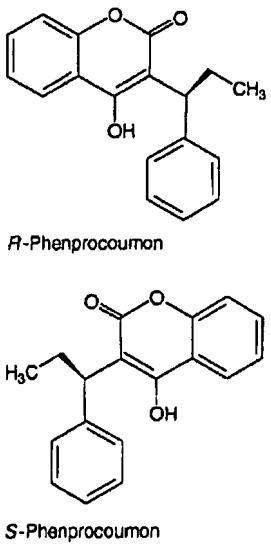


Fig. 17. The absolute configuration of *R*-phenprocoumon and *S*-phenprocoumon.

3.3 Other Pharmacokinetic Considerations

Many chiral drugs are marketed as racemates. In such cases, in fact, 2 separate drugs are being given at the same time, each with potentially different pharmacodynamics and pharmacokinetics.[83] This may not be desirable for some racemates, particularly if therapeutic and unwanted properties reside in different enantiomers. Enantioselective pharmacokinetics and disposition studies are essential to show the relative exposure of the body to the enantiomers after administration of various optical forms. Therefore, the pharmacological properties of each of the enantiomers of a racemic mixture should be evaluated to see if the risk/benefit ratio of the mixture can be improved by the use of only one of the enantiomers. Stereoselectivity in drug activity is primarily a function of differences in intrinsic activity between the isomer moieties. Stereoselective differences in drug absorption, distribution and elimination produce isomer ratios in the body that are usually different from unity. The isomeric plasma concentration ratios vary markedly from one drug to another. Preclinical development of racemic drugs should explore the pharmacological characteristics of each isomer separately as well as together.

4. Stereoselective Drug-Drug Interactions

There are more than 500 drugs currently marketed as racemates with negligible information available on the pharmacological activity, pharmacokinetic profile, metabolism or potency of individual stereoisomers.[95] Stereoselective metabolism and transport of drugs are common. Thus, drug interactions affecting biotransformation or transport may also be stereoselective. Drug-drug interactions involving stereochemical mechanisms have not received much attention to date. Drugs may interact pharmacodynamically or pharmacokinetically.[96,97] One drug may profoundly modify the action of another.

In general, the mechanisms of chiral drug interactions are as follows:[98]

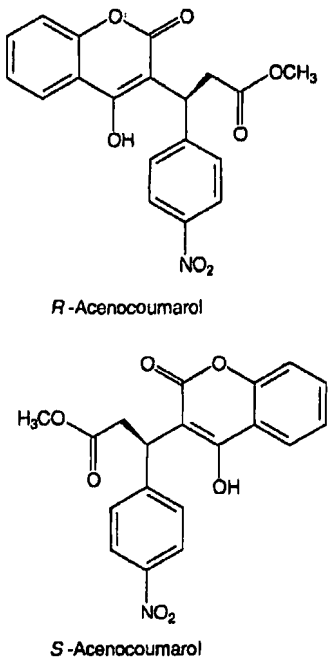


Fig. 18. The absolute configuration of *R*-acenocoumarol and *S*-acenocoumarol.

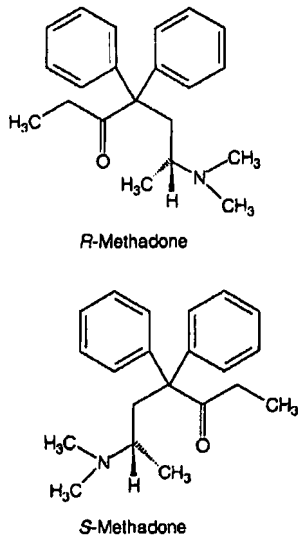


Fig. 19. The absolute configuration of *R*-methadone and *S*-methadone.

- Pharmacokinetic interactions: drugs may affect the absorption, distribution, metabolism or excretion of other drugs.[99,100]
- Pharmacodynamic interactions: drugs may have additive or synergistic pharmacological effects resulting in untoward effects. Conversely, drugs may have antagonistic pharmacological effects.[101]
- Miscellaneous: some interactions cannot be assigned to either of the above classifications.

The type of interaction between enantiomers is important in understanding both the pharmacodynamics and pharmacokinetics of chiral drugs.

Chiral drugs may interact with their own stereoisomers. For example, carbenicillin is a semisynthetic penicillin clinically used as a mixture of 2 diastereoisomers which differ only in the absolute configuration of the side chain chiral centre. Both *R,S,S,R*- and *S,S,S,R*-carbenicillin (fig. 22) bind to at least 3 binding sites of human serum albumin, one of which favours the *S,S,S,R*- and *R,S,S,R*-distereoisomer in 1 : 4 ratio. Both distereoisomers appear to displace each other at both binding sites (I and II), leading to a competitive diastereoisomer-diastereoisomer interaction that is pharmacokinetic in nature.[102] The plasma concentration of both *R*- and *S*-flurbiprofen seem to increase when each individual enantiomer was administered in humans and rats.[103]

Warfarin is an oral anticoagulant available as a racemate and is a more complex example. It is well-known for binding to site I and is also reported to exhibit stereoselective binding, so that competitive enantiomer-enantiomer interactions occur via its binding to human serum albumin.[104,105] The elimination of warfarin (fig. 23) via oxidative and reductive pathways is also stereoselective,[106] and can be altered by cimetidine, a potent inhibitor of the microsomal mixed-function oxidase system.[107] Cimetidine inhibits warfarin metabolism in a stereospecific manner, decreasing the rate of metabolism of *R*-warfarin resulting in its accumulation and having little effect on the active *S* enantiomer.[108] Furthermore, *R*-warfarin inhibits the hydroxylation of *S*-warfarin.[109] The elevated concentrations of *R*-warfarin further suppress the metabolism of *S*-warfarin leading to its accumulation and increased anticoagulant activity. Thus, warfarin exhibits enantiomer-enantiomer interactions in addition to having its elimination altered in a stereospecific manner by cimetidine. There appear to be at least 2 distinct isozymes of human hepatic cytochrome P450 (CYP) responsible for the formation of 6- and 7-hydroxy-warfarin.[110] One isozyme oxidises *R*-warfarin to *R*-6-hydroxy-warfarin and *R*-7-hydroxy-warfarin, and the other isozyme oxidises *S*-warfarin to form *S*-7-hydroxy-warfarin and *S*-6-hydroxy-warfarin. Cimetidine is found to inhibit only the isozyme which metabolises the *R* enantiomer.

Table I. Differential clearance of enantiomers of some enantiomeric drugs

Enantiomeric drug	Clearance
Betaxolol[73]	No difference between enantiomers
Carvedilol[74]	*S* > *R*
Disopyramide[75]	*S* > *R*
Ketamine[76]	*S* > *R*
Ketoprofen[77]	*R* > *S*
Salbutamol[67]	*S* < *R*

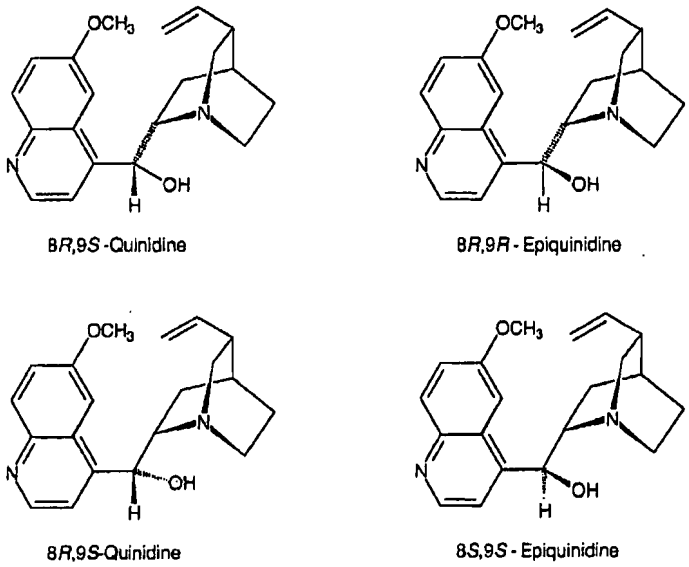


Fig. 20. The absolute configuration of 8*R*,9*S*-quinidine, 8*R*,9*R*-epiquinidine, 8*S*,9*R*-quinidine and 8*S*,9*S*-epiquinidine.

Quinidine, but not its stereoisomer quinine, inhibits the metabolism of a wide range of drugs such as propranolol and flecainide.[111,112] This is a result of the higher affinity between quinidine and CYP2D6. CYP2D6 mediates the metabolism of many drugs, including propranolol. Quinidine inhibits the elimination of both enantiomers of propranolol, but not to the same extent.[111] It also reduces the clearance of (–)-propranolol by 53% and the clearance of (+)-propranolol by 36%.

The administration of a racemate may be considered the equivalent of giving 2 different structurally related drugs, and, therefore, creating the possibility of a drug-drug interaction. Propafenone, an antiarrhythmic agent, is used chemically as a racemate, but the β-blocking activity resides almost wholly in the *S* enantiomer.[113] Therefore, the disposition of the individual enantiomers is clinically relevant. Giving *S*- and *R*-propafenone separately results in the *S* form being cleared more rapidly than the *R* form. On the other hand, the *R* enantiomer is cleared more rapidly than the *S* enantiomer when the racemate is administered. These findings suggest inhibition of metabolism of one enantiomer by the other.[114] In human liver microsomes, in the presence of the optical antipode the hydroxylation rate for *S*-propafenone decreased by 70% and that for the *R* enantiomer by 40%.[115] The more pronounced inhibition exerted by the *R* enantiomer suggests that there is a greater affinity between *R*-propafenone and the hydroxylase enzyme than between the enzyme and *S*-propafenone, and that *R*-propafenone inhibits the metabolism of *S*-

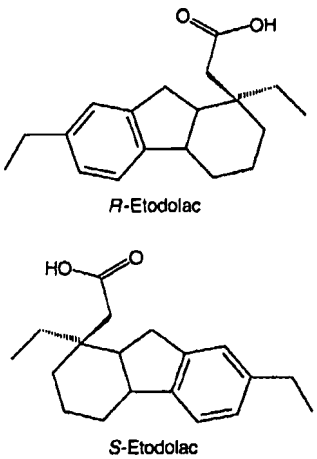
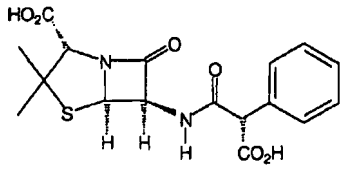
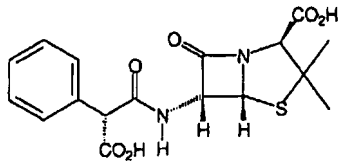


Fig. 21. The absolute configuration of *R*-etodolac and *S*-etodolac.



S,S,S,R-Carbenicillin



R,S,S,R-Carbenicillin

Fig. 22. The absolute configuration of *R,S,S,R*-carbenicillin and *S,S,S,R*-carbenicillin.

propafenone *in vitro*.[116] The adverse effect related to β-blockade in intolerant patients seems to be more evident after the administration of the propafenone racemate than the *S*-enantiomer alone.[117] Thus, the effect of racemic drug therapy is not necessarily that predicted by summation of the effects of the individual enantiomers.

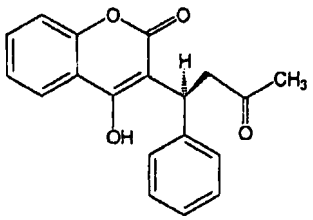
5. Enantiomeric Drugs Analysis

One major role for the chromatographic analysis of enantiomeric composition is measuring the true pharmacokinetic parameters of individual stereoisomers. Such measurements are particularly important in relating pharmacokinetic parameters to pharmacological effects. Many chiral drugs exhibit enantioselective action at target receptors or enzymes that may be magnified or dampened by the enantioselectivity of their pharmacokinetic behaviour.[116,118] For chromatographic discrimination, the phenomenon appears between the chiral stationary phase and the individual enantiomer. Thus, there is a need for stereospecific detection, identification and quantification of individual enantiomers of drugs and their metabolites in various biological media. The concentration of these analytes can often be as low as a few nanograms per millilitre in complex biological fluids containing many other substances.

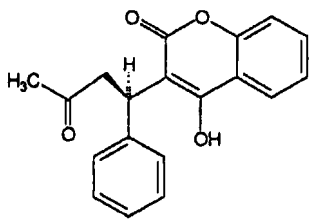
Enantiomeric discrimination can be achieved with different chromatographic techniques. Most assays developed to control the determination of drug enantiomers use chiral high performance liquid chromatography (HPLC).[119-121] Other chromatographic methods include capillary electrophoresis[122] and capillary electro-osmosis.[123] The principal feature of chiral enantioseparation is that one enantiomer is immobilised on liquid chromatographic support more strongly than the other. For this to occur there must be at least 3 simultaneous interactions between a chiral stationary phase and a single enantiomer of drug.[124-127] The preferential interactions include π-π interactions, hydrogen bonding, and dipole to dipole hydrophobic or steric effects. One of these interactions must be dependent on the stereochemical structure of the chiral drug.

6. Enantiomeric Drug Appraisal

Enantioselective pharmacokinetics, drug-drug interactions and drug disposition studies are essential to provide an understanding of mechanisms of drug action in biological systems after administration of various optical forms. It is possible that for many racemic drugs the adverse effects of an un-



S-(–)-Warfarin



R-(+)-Warfarin

Fig. 23. The absolute configuration of *S*-(–)-warfarin and *R*-(+)-warfarin.

desirable enantiomer will go undetected. However, on the basis of racemic drugs studied to date, it should be assumed that enantiomorphs have differences in quantitative and/or qualitative activity until proven otherwise.

In this context it may be considered that racemates contain up to 50% impurity – a situation that would usually be considered undesirable.[128] Changes in the enantiomeric composition of chiral drugs may result in alterations in their pharmacological and toxicological profiles, i.e. the 'inactive' enantiomer may directly or indirectly contribute to the overall therapeutic profile of the racemate. Hence, for existing racemic drugs with satisfactory safety records, the optically pure drug does not necessarily provide better medication. However, dismissing the potential advantages of single enantiomers based on what is known about the racemate is also unwarranted, as single enantiomers may provide new therapeutic opportunities. Therefore, stereochemically pure compounds should be considered as new drugs. From a research and development viewpoint they must be investigated thoroughly for possible additional indications and adverse effects in comparison with their respective racemic formulations.[125-127]

It is also important that the issue of enantiomer *vs* racemate is examined on the basis of individual drugs, since guidelines cannot be provided for entire classes of drugs. The decision to develop a stereochemical pure compound must be based on pharmacodynamic, safety, pharmacokinetic and pharmaceutic considerations. Table II shows a number of reasons for development of a single enantiomer and the justification for the development of a racemate.[95]

The concept of '50% impurity' is not applicable to many drugs in widespread use. There are few examples of enantiomers with different pharmacological and pharmacokinetic properties and while their potency may differ greatly, at achievable doses the pharmacological actions of the racemate are usually the same as the actions of the more potent enantiomer. Some examples include ketamine, in which the *S*-(+) isomer is more potent,[129]

Table II. Justification for development of single enantiomers and racemates

Single enantiomer	Racemate
Therapeutic activity may reside primarily in one enantiomer	Additive or synergistic therapeutic activities of both enantiomers
More specific effect	High therapeutic index
Fewer or less severe adverse reactions	Negligible/low toxicity of the diastereoisomer
Greater potency of one enantiomer	Chiral inversion yields fixed ratio of enantiomers
No chiral inversion	Physicochemical characteristics favouring use of racemate
Blood concentration measurement of active drug is simpler • clearer pharmacokinetics • better for therapeutic drug monitoring	Novel drug, chiral properties not well defined
Economic feasibility	Indication for life-threatening disease
	Not cost effective to manufacture active enantiomer on a large scale

and warfarin,[130] where the pharmacokinetics and related potency of the enantiomers are different.[131,132] Yet, even with all of this information, there has been no evidence that giving one enantiomer would lead to better therapeutics than the present practice of giving the racemate.

Thus, there is a need for stereospecific detection, identification and quantification of individual enantiomers of drugs and their metabolites in various biological media. Even if a drug is given as a pure enantiomer, methods that can discriminate between enantiomers will be required because racemisation can occur both *in vitro*[133] and *in vivo*.[134] *R*-Ketoprofen racemises after 30 minutes of incubation with mitochondrial fraction. The epimeric composition of the resulting ketoprofenyl-CoA was 52.3% *R* enantiomer and 47.7% *S* enantiomer.[135] To avoid adverse effects and to optimise the therapeutic value of the drug, methods must evolve and be devolved to determine isomeric purity, establish the effectiveness of chiral compounds of a drug and detect the presence of enantiomers

with lower therapeutic activity or more adverse effects.[128]

The ideal preclinical and clinical development of racemic drugs should explore the pharmacological characteristics of the isomers separately and together. Considering the separate isomers as the same chemical entity may be misleading or harmful. With genetic engineering to assist synthetic chemistry, complex structures with a high degree of stereospecificity are commonly being developed. A single acting moiety remains the preferred goal, but it is overly simplistic to say that only single enantiomers should be candidates for future drugs.

References

1. De Camp WH. FDA perspective on the development of new stereoisomeric drugs: chemistry, manufacturing and control issues. In: Wainer IW, editor. Drug stereochemistry: analytical methods and pharmacology. 2nd ed. New York: Marcel Decker, Inc., 1993: 365-84
2. Rauws AG, Groen K. Current regulatory guidance on chiral medicinal products: Canada, EEC, Japan and US. Chirality 1994; 6: 72-5
3. Tomaszewski J, Rumore MM. Stereoisomeric drugs: FDA's policy statement and the impact on drug development. Drug Dev Indust Pharm 1994; 20: 119-39
4. Stinson SC. Chiral drugs. Chem Eng News 1992; 70: 41-79
5. McMahon FG, Achecar FA, Murray MJ, et al. The importance of chirality to antihypertensive drug effects. Curr Ther Res Clin Exp 1992; 52: 89-97
6. Serilevy A, West S, Richards WG. Molecular similarity, quantitative chirality, and QSAR for chiral drugs. J Med Chem 1994; 37: 1727-32
7. Walther W, Netscher T. Design and development of chiral drugs for the chromatography of chiral alcohols. Chirality 1996; 8: 397-401
8. Gorog S, Gazdag M. Enantiomeric derivatization for biomedical chromatography. J Chromatogr B-Biomed Appl 1994; 659: 51-84
9. Ariens EJ. Stereochemistry in the analysis of drug action. Part II. Med Res Rev 1987; 7: 367-87
10. Ariens EJ. Implications of the neglect of stereochemistry in pharmacokinetics and clinical pharmacology. Drug Intell Clin Pharm 1987; 21: 827-9
11. Holmstedt B, Frank H, Testa B. Chirality and biological activity. New York: Liss, 1990
12. Walle UK, Pesola GR, Walle T. Stereoselective sulfate conjugation of salbutamol in humans: comparison of hepatic, intestinal and platelet activity. Br J Clin Pharmacol 1993; 35: 413-8
13. Piotrovskij VK, Shvatchko EV, Trnovec T. The use of physiological-based models to simulate enantioselective differences in pharmacokinetics. Methods Find Exp Clin Pharmacol 1994; 16: 263-9
14. Easson LH, Stedman E. Studies on the relationship between chemical constitution and physiological action. 5-Molecular dissymmetry and physiological activity. Biochem J 1993; 27: 1257-66
15. Hayball PJ. Chirality and nonsteroidal anti-inflammatory drugs. Drugs 1996; 52: 47-58
16. Hutt AJ, Tan SC. Drug chirality and its clinical significance. Drugs 1996; 52: 1-12
17. Lotsch J, Geisslinger G, Mohammadian P, et al. Effects of flurbiprofen enantiomers on pain-related chemosomatosensory evoked-potentials in human subjects. Br J Clin Pharmacol 1995; 40: 339-46
18. Motzer RJ, Lyn P, Fischer P, et al. Phase I/II trial of dexverapamil plus vinblastine for patients with advanced renal cell carcinoma. J Clin Oncol 1995; 13: 1958-65
19. Andrews JE, Ebronmccoy M, Bojic U, et al. Validation of an *in vitro* teratology system using chiral substances: stereoselective teratogenicity of 4-yn-valproic acid in cultured mouse embryos. Toxicol Appl Pharmacol 1995; 132: 310-6
20. Mcmahon FG, Achecar FA, Murray MJ, et al. The importance of chirality to antihypertensive drug effects. Curr Ther Res 1992; 52: 89-97
21. Ariens EJ, Wuis EW. Bias in pharmacokinetics and clinical pharmacology. Clin Pharmacol Therapeut 1987; 42: 361-3
22. Walle T, Walle UK. Chirality Part 4. Pharmacokinetic parameters obtained with racemate. Trends Pharmacol Sci 1986; 7: 115-58
23. Pendleton RG, Newman DJ, Sherman SS, et al. Effect of propranolol upon the hemoglobin-oxygen dissociation curves. J Pharmacol Exp Ther 1972; 180: 647-50
24. Oski FA, Miller LD, Delivoria-Papadopoulos M, et al. Oxygen affinity in the red cells. Changes induced *in vivo* by propranolol. Science 1972; 175: 1372-3
25. Campbell RWF, Murray A, Julian DG. Ventricular arrhythmias in first 12 hours of acute myocardial infarction. Br Heart J 1981; 46: 351-7
26. Wilson AG, Brooke OG, Lloyd HF, et al. Mechanism of action of β-adrenergic receptor blocking agents in angina pectoris: comparison of action of propranolol with dexpropranolol and practolol. BMJ 1969; 4: 399-401
27. Brittain RT, Drew GM, Levy GP. The α-adrenoceptor and β-adrenoceptor blocking potencies of labetalol and its individual stereoisomers in anesthetized dogs and in isolated tissues. Br J Pharmacol 1982; 77: 105-14
28. Byrnes EW, McMaster PD, Smith ER, et al. New antiarrhythmic agents. 1. Primary a-amino anilides. J Med Chem 1979; 22: 1171-6
29. Doenicke A, Kugler J, Mayer M, et al. Influence of racemic ketamine and *S*-(+)-ketamine on vigilance, performance and wellbeing. Anaesthetist 1992;41: 610-8
30. Powell JR, Ambre JJ, Ruo TI. Drug stereochemistry: analytical methods and pharmacology. In: Wainer IW, Drayer DE, editors. New York: Marcel Dekker, Inc., 1988: 245-70
31. Downes H, Perry RS, Ostlund RE, et al. Excitatory effects of barbiturates. J Pharmacol Exp Ther 1970; 175: 692-9
32. Buech HP, Schneider-Affeld F, Rummel W. Stereochemical dependence of pharmacological activity in a series of optically active *N*-methylated barbiturates. Naunyn Schmiedebergs Arch Pharmacol 1973; 277: 191-8
33. Waddell WJ, Baggett B. Anesthetic and lethal activity in mice of the stereoisomers of 5-ethyl-5-methylbutyl barbituric acid (pentobarbitol). Arch Int Pharmacodyn Therapie 1973; 205: 40-4
34. Ambre JJ, Fisher LJ. Glutethimide intoxication: plasma levels of glutethimide and a metabolite in humans, dogs and rats. Res Comm Chem Pathol Pharmacol 1972; 4: 307-26

35. Ambre JJ, Fisher LJ. Identification and activity of hydroxy-metabolite that accumulates in plasma of humans intoxicated with glutethimide. Drug Metab Dispos 1974; 2: 151-8
36. Hansen AR, Ambre JJ, Fischer LJ. Glutethimide poisoning: a metabolite contributes in morbidity and mortality. N Engl J Med 1975; 292: 250-2
37. Hansen AR, Kennedy KA, Ambre JJ, et al. Activity of glutethimide metabolite in human beings. N Engl J Med 1975; 292: 871-3
38. Hansen AR, Kennedy KA, Ambre JJ, et al. Gluthethimide poisoning: a metabolite contributes to morbidity and mortality. N Eng J Med 1975; 292: 250-2
39. Reddy IK, Korlipara VL. Chirality implications on drug actions. US Pharmacist 1995; 20: H20-4
40. Scott AK. Stereoisomers and drug toxicity: the value of single stereoisomer therapy. Drug Saf 1993; 8: 149-59
41. Wilson JL, Nayanar V, Walker JS. The site of antiarthritic action of the kappa-opioid, U-50, 488H, in adjurant arthritis: importance of local administration. Br J Pharmacol 1996; 118: 1754-60
42. Geisslinger G, Schnible HG. New insight into the site and mode of antinociceptive action of flurbiprofen enantiomers. J Clin Pharmacol 1996; 36: 513-20
43. Kharasch ED, Herrmann S, Labroo R. Ketamine as a probe for medetomidine stereoisomer inhibition of human liver microsomal drug metabolism. Anesthesiology 1992; 77: 1208-14
44. Yang T, Prakash C, Roden DM, et al. Mechanism of block of a human cardiac potassium channel by terfenadine racemate and enantiomers. Br J Pharmacol 1995; 115: 267-74
45. Toldy L, Vargha L, Toth I, et al. Promethazine. I. Acta Chim Acad Sci Hung 1959; 19: 273-5
46. Breckenridge A, Orme M, Wesseling H, et al. Pharmacokinetics and pharmacodynamics of the enantiomers of warfarin in man. Clin Pharmacol Ther 1974; 15: 424-30
47. Palareti G, Legnani C. Warfarin withdrawal: pharmacokinetic-pharmacodynamic consideration. Clin Pharmacokinet 1996; 30: 300-13
48. Satoh K, Yanagisawa T, Taira N. Coronary vasodilator and cardiac effects of optical isomers of verapamil in the dog. J Cardiovasc Pharmacol 1980; 2: 309-18
49. Mehver R, Reynolds JM, Robinson MA, et al. Enantioselective kinetics of verapamil and nonverapamil in isolated perfused rat livers. Pharm Res 1994; 11: 1815-9
50. Barrett AM, Cullum VA. The biological properties of the optical isomers of propranolol and their effects on cardiac arrhythmias. Br J Pharmacol Chemo 1968; 34: 43-55
51. Boyd GS, Oliver MF. Thyroid hormones and plasma lipids. Br Med Bull 1960; 16: 138-41
52. Arends RHGP, Zhang KY, Levy RH, et al. Stereoselective pharmacokinetics of stiripentol: an explanation for the development of tolerance to anticonvulsant effect. Epilepsy Res 1994; 18: 91-6
53. Graf BM, Vicenzi MN, Martin E, et al. Ketamine has stereospecific effects in the isolated perfused guinea pig heart. Anesthesiology 1995; 82: 1426-37
54. Mathisen LC, Skjelbred P, Skoglund LA, et al. Effect of ketamine, an NMDA receptor inhibitor, in acute and chronic orofacial pain. Pain 1995; 61: 215-20
55. White PF, Ham J, Way WL, et al. Pharmacology of ketamine isomers in surgical patients. Anesthesiology 1980; 52: 231-9
56. Doenicke A, Angster R, Mayer M, et al. Hemodynamics, plasma-catecholamine and cortisol levels after ketamine racemate and S-(+)-ketamine. Anaesthetist 1992; 41: 597-603
57. Prichard BNC. Combined α- and β-adrenoceptor inhibition in the treatment of hypertension. Drugs 1984; 28: 51-68
58. Hartigango K, Bateman DN, Daly AK, et al. Stereoselective cardiotoxic effects of terodiline. Clin Pharmacol Ther 1996; 60: 89-98
59. Gumbleton M, Forbes BJ. Principles in the absorption, distribution and elimination of pharmaceuticals. Pestic Sci 1994; 42: 223-40
60. Valenzuela C, Snyders DJ, Bennett PB, et al. Stereoselective block of cardiac sodium channels by bupivacaine in guinea pig ventricular myocytes. Circulation. 1995; 92: 3014-24
61. Wainer IW. Stereoisomers in clinical oncology: why it is important to know what the right and left hands are doing. Ann Oncol 1993; 4: S7-13
62. Lacthem ME, Belpaire FM, Wijnant P, et al. Stereoselective pharmacokinetics of oxprenolol, propranolol, and verapamil: species differences and influence of endotoxin. Chirality 1995; 7: 616-22
63. Pacifici GM, Desanti C, Mussi A, et al. Interindividual variability in the rate of salbutamol sulfation in the human lung. Eur J Clin Pharmacol 1996; 49: 299-303
64. Eaton EA, Walle UK, Wilson HM, et al. Stereoselective sulphate conjugation of salbutamol by human lung and bronchial epithelial cells. Br J Clin Pharmacol 1996; 41: 201-6
65. Chapman ID, Buchheit KH, Manley P, et al. Active enantiomers may cause adverse effects in asthma. Trends Pharmacol Sci 1992; 13: 231-35
66. Pesola GR, Walle T. Enatiomeric interaction in the sulfate conjugation of the β2-agonist drug albuterol by the human liver. Res Commun Chem Pathol Pharmacol 1992; 75: 125-8
67. Boulton DW, Fawcett JP. Enantioselective disposition of salbutamol in man following oral and intravenous administration. Br J Clin Pharmacol 1996; 41: 35-40
68. Drayer DE. Pharmacodynamics and pharmacokinetics differences between drug enantiomers in humans: an overview. Clin Pharmacol Ther 1986; 40: 125-33
69. Bourahla A, Martin C, Gimenez F, et al. Stereoselective pharmacokinetics of mefloquine in young children. Eur J Clin Pharmacol 1996; 50: 241-4
70. Walle T, Webb JG, Bagwell EE, et al. Stereoselective delivery and actions of β-receptor antagonists. Biochem Pharmacol 1988; 37: 115-24
71. Fehske KJ, Muller E, Woller U. The location of drug binding sites in human serum albumin. Biochem Pharmacol 1981; 30: 687-92
72. Kristensen K, Blemmer T, Angelo HR, et al. Stereoselective pharmacokinetics of methadone in chronic pain patients. Ther Drug Monit 1996; 18: 221-7
73. Stagni G, Davis PJ, Ludden TM. Human pharmacokinetics of betaxolol enantiomers. J Pharm Sci 1991; 80: 321-4
74. Zhou HH, Wood AJJ. Stereoselective disposition of carvedilol is determined by CYP2D6. Clin Pharmacol Ther 1995; 57: 518-24
75. Echizen H, Takahashi H, Nakamura H, et al. Stereoselective disposition and metabolism of disopyramide in pediatric patients. J Pharmacol Exp Ther 1991; 259: 953-60
76. Kharasch ED, Labroo R. Metabolism of ketamine stereoisomers by human liver microsomes. Anesthesiology 1992; 77: 1201-7
77. Skeith KJ, Russell AS, Jamali F. Ketoprofen pharmacokinetics in the elderly; influence of rheumatic disease, renal function, and dose. J Clin Pharmacol 1993; 33: 1052-9

78. Notterman DA, Drayer DE, Metakis L, et al. Stereoselective renal tubular secretion of quinidine and quinine. Clin Pharmacol Ther 1986; 40: 511-7
79. Echizen H, Vogelgesang B, Eichelbaum M. Effect of d,l-verapamil on atrioventricular conduction in relation to its stereoselective first-pass metabolism. Clin Pharmacol Ther 1985; 38: 71-6
80. Von Bahr C, Hermansson J, Tawara K. Plasma levels of (+)- and (−)-propranolol and 4-hydroxypropranolol after administration of racemic (±)-propranolol in man. Br J Clin Pharmacol 1982; 14: 79-82
81. Sedman AJ, Gal J, Mastropaolo E, et al. Serum tocainide enantiomer concentrations in human subjects. Br J Clin Pharmacol 1984; 17: 113-5
82. Thomson AB, Murdock G, Pottage A, et al. Pharmacokinetics of (*R*)- and (*S*)-tocainide in patients with ventricular arrhythmias. Br J Clin Pharmacol 1986; 21: 149-54
83. Drayer DE. Stereochemistry: analytical methods and pharmacology. In: Wainer IW, Drayer DE, editors. New York: Marcel Dekker, Inc., 1988: 209-25
84. Thijssen HHW, Janssen GMJ, Baars LGM. Lack of effect of cimetidine on pharmacodynamics and kinetics of single oral does of (*R*)-acenocoumarol and (*S*)-acenocoumarol. Eur J Clin Pharmacol 1986; 30: 619-23
85. Tucker GT, Lennard MS. Enantiomer-specific pharmacokinetics. Pharmacol Ther 1990; 45: 309-29
86. Drayer DE. Problems in therapeutic drug monitoring: the dilemma of enantiomeric drugs in man. Ther Drug Monit 1988; 10: 1-7
87. McMenany R, Oncley JL. Specific binding of l-tryptophan to serum albumin. J Biol Chem 1958; 233: 1436-47
88. Muller WE, Wollert U. High stereospecificity of the benzodiazepine binding site on human serum albumin. Mol Pharmacol 1975; 11: 52-60
89. Brown NA, Jahnchen E, Muller WE, et al. Optical studies on the mechanism of the interaction of the enantiomers of the anticoagulant drugs phenprocoumon and warfarin with human serum albumin. Mol Pharmacol 1977; 13: 70-9
90. Dayton P, Stiller RL, Cook DR, et al. Binding of ketamine to plasma proteins: emphasis on human plasma. Eur J Clin Pharmacol 1983; 24: 825-31
91. Walle UK, Walle T, Bai SA, et al. Stereoselective binding of propranolol to human plasma, α_1-acid glycoprotein and albumin. Clin Pharmacol Ther 1983; 34: 718-23
92. Mignot I, Presle N, Lapicque F, et al. Albumin binding sites for etodolac enantiomers. Chirality 1996; 8: 271-80
93. Brocks DR, Jamali F, Russel AF. Stereoselective disposition of etodolac enantiomers in synovial fluid. J Clin Pharmacol 1991; 31: 741-6
94. Muller N, Lapicque F, Monot C, et al. Stereoselective binding of etodolac to human serum albumin. Chirality 1992; 4: 240-6
95. Cayen MN. Racemic mixtures and single stereoisomers: industrial concerns and issues in drug development. Chirality 1991; 3: 94-8
96. Pesola GR, Walle T. Enantiomeric interaction in the sulfate conjugation of the β2-agonist drug albuterol by the human liver. Res Commun Chem Patho Pharmacol 1992; 75: 125-8
97. Brocks DR, Jamali F. Stereochemical aspects pf pharmacotherapy. Pharmacotherapy 1995; 15: 551-64
98. Hansten PD. Drug interactions: clinical significance of drug-drug interactions and drug effects on clinical laboratory results. Philadelphia: Lea and Febiger, 1975
99. Berry, Jamali. Enantiomeric interaction of flurbiprofen in the rat. J Pharm Sci 1989; 78: 632-4
100. Li G, Treiber G, Klotz U. Ibuprofen-cimetidine interaction: stereochemical considerations. Drug Invest 1989; 1: 11-7
101. Wingard LB, Levy G. Prothrombin complex activity produced equieffective doses of (R)-(+)- and (S)-(−)-warfarin in humans. J Pharm Sci 1997; 66: 1790-1
102. Itoh T, Nakashima K, Tsuda Y, et al. Stereoselective binding of carbenicillin epimers to human serum albumin. Chirality 1996; 8: 201-6
103. Jamali F. Stereochemically pure drugs: an overview. In: Wainer IW, editor. Drug stereochemistry: analytical methods and pharmacology. 2nd ed. New York: Marcel Dekker, Inc, 1993: 375-84
104. Yacobi A, Levy G. Protein binding of warfarin enantiomers in serum of humans and rats. J Pharmacokinet Biopharm 1977; 5: 123-31
105. Loun B, Hage DS. Chiral separation mechanisms in protein-based HPLC columns. 1. Thermodynamic studies of (*R*)- and (*S*)-warfarin binding to immobilised human serum albumin. Anal Chem 1994; 66: 381-2
106. Lewis RJ, Trager WF, Chan KK, et al. Warfarin: stereochemical aspects of its metabolism and the interaction with phenylbutazone. J Clin Invest 1974; 53: 1607-17
107. Somogyi A, Gugler R. Drug interactions with cimetidine. Clin Pharmacokinet 1982; 7: 23-41
108. Choonara IA, Cholerton S, Haynes BP, et al. Stereoselective interaction between the (*R*)-enantiomer of warfarin and cimetidine. Br J Clin Pharmacol 1986; 21: 271-7
109. Kunze KL, Eddy AC, Gribaldi M, et al. Metabolic enantiomeric interactions: the inhibition of human *S*-warfarin-7-hydroxylase by *R*-warfarin. Chirality 1991; 3: 24-9
110. Niopas I, Toon S, Rowland M. Further insight into the stereoselective interaction between warfarin and cimetidine in man. Br J Pharmacol 1991; 32: 508-11
111. Zhou HH, Anthony LB, Reden DM, et al. Quinidine reduces clearance of (+)-propranolol more than (−)-propranolol through marked reduction in 4-hydroxylation. Clin Pharmacol Ther 1990; 47: 686-93
112. Birgersdotter UM, Wong W, Turgeon J, et al. Stereoselectivity genetically determined interaction between chronic flecainide and quinidine in patients with arrhythmias. Br J Clin Pharmacol 1992; 33: 275-80
113. Kroemer HK, Funck-Brentane C, Silberstein DJ, et al. Stereoselective disposition and pharmacological activity of propafenone enantiomers. Circulation 1989; 79: 1068-76
114. Brode E, Muller H, Peltzer O, et al. Comparative pharmacokinetics and clinical pharmacology of propafenone enantiomers after oral administration to man. Methods Find Exp Clin Pharmacol 1988; 10: 717-27
115. Kroemer HK, Fischer C, Meese CO, et al. Enantiomer/enantiomer interaction of (*S*)- and (*R*)-propafenone for cytochrome P450IID6-catalyzed 5-hydroxylation: *in vitro* evaluation of mechanism. Mol Pharmacol 1991; 40: 135-42
116. Kroemer HK, Fromm MF, Buhl K, et al. An enantiomer-enantiomer interaction of (*S*)-propafenone and (*R*)-propafenone modifies the effect of racemic drug-therapy. Circulation 1994; 89: 2396-400
117. Zily W, Breimer DD and Richter E. Induction of drug metabolism in man after rifampicin treatment measured by increased hexobarbital and tolutamide clearance. Eur J Clin Pharmacol 1975; 9: 219-27
118. Caldwell J. Racemates and enantiomers: scientific and regulatory aspects. Chirality 1989; 1: 249-50
119. Abul-Enein HY, Islam MR. Direct HPLC separation of thalidomide enantiomers using cellulose tris-4-methylphenyl ben-

zoate chiral stationary phase. J Liqu Chromatog 1991; 14: 667-73
120. Yashima E, Sahavattanapong P, Okamoto Y. HPLC enantioseparation on cellulose Tris(3,5-dimethylphenylcarbamate) as a chiral stationary phase: influences of pore size of silica gel, coating amount, coating solvent and column temperature on chiral discrimination. Chirality 1996; 8: 446-51
121. Baume B, Fernandez C, Gimenez F, et al. Chirality: pharmacokinetic implications. Part I. Methods of separation of isomers of chiral drugs: application to the determination of their concentration in biological fluids. J Pharm Clinique 1992; 11: 81-94
122. Shaw CJ, Silverman CE. Enantiomeric separation of aminophosphonic acids by capillary electrophoresis. Chirality 1996; 8: 84-7
123. Ward TJ, Dann III C, Brown AP. Separation of enantiomers using vancomycin in a countercurrent process by suppression of electro-osmosis. Chirality 1996; 8: 77-83
124. Dalgliesh CE. Optical resolution of aromatic amino acids on paper chromatography. J Chem Soc 1952; 137: 3940-2
125. Wainer IW, Caldwell J. The 1996 chirality debate. Chirality 1997; 9: 95
126. Booth TD, Wahnon D, Wainer IW. Is chiral recognition a three-point process? Chirality 1997; 9: 96-8
127. Davankov VA. The nature of chiral recognition: is chiral a three-point interaction? Chirality 1997; 9: 99-102
128. Ariens EJ. Stereochemistry, a basis for sophisticated nonsense in pharmacokinetics and clinical pharmacology. Eur J Clin Pharmacol 1984; 26: 663-8
129. White PF, Schutter J, Shafer A, et al. Comparative Pharmacology of the ketamine isomers. Br J Anaesth 1985; 57: 197-203
130. Lewis RJ, Trager WF. Warfarin metabolism in man: identification of metabolites in urine. J Clin Invest 1970; 49: 907-13
131. Lewis RJ, Trager WF, Chan KK, et al. Warfarin: stereochemical aspects of its metabolism and interaction with phenylbutazone. J Clin Invest 1974; 53: 1607-17
132. Serlin MJ, Breckenridge AM. Drug interactions with warfarin. Drugs 1983; 25: 610-20
133. Eliel EL. Stereochemistry of carbon compounds. New York: McGraw-Hill, 1962
134. Testa B. The chromatographic analysis of enantiomers in drug metabolism studies. Xenobiotica 1986; 16: 265-79
135. Carabaza A, Suesa N, Tost D, et al. Stereoselective metabolic pathways of ketoprofen in the rat: incorporation into triacylglycerols and enantiomeric inversion. Chirality 1996; 8: 163-72

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